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(54) Title: PHARMACEUTICAL COMPOSITION COMPRISING A BISPECIFIC ANTIBODY FOR EPCAM

(57) Abstract: The present invention provides a pharmaceutical composition comprising a bispecific single chain antibody construct. Said bispecific single chain antibody construct is characterized to comprise or consist of at least two domains, whereby one of said at least two domains specifically binds to human EpCAM and comprises at least one CDR-H3 region comprising the amino acid sequence NXID antigen and a second domain binds to human CD3 antigen. The invention further provides a process for the production of the pharmaceutical composition of the invention, a method for the prevention, treatment or amelioration of a tumorous disease and the use of the disclosed bispecific single chain antibody construct and corresponding means in the prevention, treatment or amelioration of a tumorous disease.

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**PHARMACEUTICAL COMPOSITION COMPRISING  
A BISPECIFIC ANTIBODY SPECIFIC FOR EPCAM**

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The invention relates to a pharmaceutical composition comprising a bispecific single chain antibody construct. Said bispecific single chain antibody construct is characterized to comprise or consist of at least two domains, whereby one of said at least two domains specifically binds to human EpCAM antigen and comprises at least one CDR-H3 region comprising the amino acid sequence NXD and a second domain binds to human CD3 antigen. The invention further provides a process for the production of the pharmaceutical composition of the invention, a method for the prevention, treatment or amelioration of a tumorous disease and the use of the disclosed bispecific single chain antibody construct and corresponding means in the prevention, treatment or amelioration of a tumorous disease.

A variety of documents is cited throughout this specification. The disclosure content of said documents is herewith incorporated by reference.

Epithelial cell adhesion molecule (EpCAM, also called 17-1A antigen, KSA, EGP40, GA733-2, ks1-4 or esa) is a 40-kDa membrane-integrated glycoprotein of 314 amino acids with specific expression in certain epithelia and on many human carcinomas (reviewed in Balzar, J. Mol. Med. 1999, 77, 699-712). EpCAM was discovered and subsequently cloned through its recognition by the murine monoclonal antibody 17-1A/edrecolomab (Goettlinger, Int J Cancer. 1986; 38, 47-53 and Simon, Proc. Natl. Acad. Sci. USA. 1990; 87, 2755-2759). Monoclonal antibody 17-1A was generated by immunization of mice with human colon carcinoma cells (Koprowski, Somatic Cell Genet. 1979, 5, 957-971).

The EGF-like repeats of EpCAM were shown to mediate lateral and reciprocal interactions in homophilic cell adhesion (Balzar, Mol. Cell. Biol. 2001, 21, 2570-2580) and, for that reason, is predominantly located between epithelial cells (Litvinov, J Cell Biol. 1997, 139, 1337-1348, Balzar, J Mol Med. 1999, 77, 699-712 and Trebak, J Biol Chem. 2001, 276, 2299-2309). EpCAM serves to adhere epithelial cells in an oriented and highly ordered fashion (Litvinov, J Cell Biol. 1997, 139, 1337-1348). Data from experiments with transgenic mice and rats expressing human EpCAM on their epithelia suggest that EpCAM on normal tissue may however not be accessible to systemically administered antibody (McLaughlin, Cancer Immunol. Immunother., 1999, 48, 303-311). Upon malignant transformation of epithelial cells the rapidly growing tumor cells are abandoning the high cellular order of epithelia. Consequently, the surface distribution of EpCAM becomes less restricted and the molecule better exposed on tumor cells. Due to their epithelial cell origin, tumor cells from most carcinomas still express EpCAM on their surface.

In vivo, expression of EpCAM is related to increased epithelial proliferation and negatively correlates with cell differentiation (for review see Balzar, 1999, J. Mol. Med. 77, 699-712). Expression of EpCAM, as detected by immunohistochemistry using anti-EpCAM monoclonal antibodies, is essentially seen with all major carcinomas (reviewed in Balzar, J Mol Med. 1999, 77, 699-712). Best EpCAM expression was observed with non-small cell lung cancer (De Bree, Nucl Med Commun. 1994, 15, 613-27) and prostate cancer (Zhang, Clin Cancer Res. 1998, 4, 295-302) where 100% of tumor patient samples showed positive EpCAM staining. In these studies, EpCAM is also reported to homogeneously stained tumor tissues indicating that the antigen is expressed on a large proportion of cells of a given tumor. Because of its widespread expression, EpCAM is referred to as a "pan-carcinoma" antigen.

EpCAM has been shown in various studies to be beneficial in diagnosis and therapy of various carcinomas. Furthermore, in many cases, tumor cells were

observed to express EpCAM to a much higher degree than their parental epithelium or less aggressive forms of said cancers. For example, EpCAM expression was shown to be significantly higher on neoplastic tissue and in adenocarcinoma than on normal prostate epithelium ( $n=76$ ;  $p<0.0001$ ), suggesting that increased EpCAM expression represents an early event in the development of prostate cancer (Poczatek, J Urol., 1999, 162, 1462-1644). In addition, in the majority of both squamous and adenocarcinomas of the cervix a strong EpCAM expression correlates with an increased proliferation and the disappearance of markers for terminal differentiation (Litvinov, Am. J. Pathol. 1996, 148, 865-75). One example is breast cancer where overexpression of EpCAM on tumor cells is a predictor of survival (Gastl, Lancet. 2000, 356, 1981-1982). Furthermore, EpCAM has been described as a marker for the detection of disseminated tumor cells in patients suffering from squamous cell carcinoma of the head, neck and lung (Chaubal, Anticancer Res 1999, 19, 2237-2242, Piyathilake, Hum Pathol. 2000, 31, 482-487). Normal squamous epithelium, as found in epidermis, oral cavity, epiglottis, pharynx, larynx and esophagus did not significantly express EpCAM (Quak, Hybridoma, 1990, 9, 377-387).

In addition to the above-mentioned carcinomas, EpCAM has been shown to be expressed on the majority of primary, metastatic, and disseminated NSCLC (non small cell lung cancer cells) (Passlick, Int J Cancer, 2000, 87, 548-552), on gastric and gastro-oesophageal junction adenocarcinomas (Martin, J Clin Pathol 1999, 52, 701-4) and in cell lines derived from colorectal, pancreatic carcinomas and breast carcinomas (Szala, Proc Natl Acad Sci U S A 1990, 87, 3542-6, Packeisen, Hybridoma, 1999, 18, 37-40).

Clinical trials have shown that the use of antibodies directed against 17-1A (EpCAM) for treatment of patients with surgically completely resected colorectal carcinoma leads to a significant benefit concerning the overall survival and the frequency of distant metastasis (Riethmüller, Lancet, 1994, 343, 1177-1183). Murine monoclonal antibody against EpCAM was found to reduce the 5-year



mortality (Riethmüller, Lancet, 1994, 343, 1177-1183) and also the 7-year mortality (Riethmüller, Proceedings of the American Society of Clinical Oncology, 1996, 15, 444) of patients with minimal residual disease. Example of murine monoclonal antibody recognizing EpCAM is Edrecolomab (Panorex) (Koprowski, Somatic Cell Genet. 1979, 5, 957-971 and Herlyn, Cancer Res., 1980, 40, 717-721). However, the first administration of Panorex during adjuvant immunotherapy of colon cancer led to the development and exacerbation of Wegener's granulomatosis suggesting that mAb 17-1A should be applied cautiously in a patient with autoimmune disease (Franz, Onkologie, 2000, 23, 472-474). The limitations of Panorex are the rapid formation of human anti-mouse antibodies (HAMA), the limited ability to interact by its murine IgG2a Fc-portion with human immune effector mechanisms and the short half-life in circulation (Frodin, Cancer Res., 1990, 50, 4866-4871). Furthermore, the murine antibody caused immediate-type allergic reactions and anaphylaxis upon repeated injection in patients (Riethmüller, Lancet. 1994, 343, 1177-1183, Riethmüller, J Clin Oncol., 1998, 16, 1788-1794 and Mellstedt, Annals New York Academy of Sciences. 2000, 910, 254-261).

Humanized anti-EpCAM antibody called 3622W94 resulted in pancreatitis and increased serum levels of amylase, as being indicative for damage of pancreas epithelium, which were a dose-limiting toxicity of this high-affinity anti-EpCAM monoclonal antibody (LoBuglio, Proceedings of the American Society of Clinical Oncology (Abstract). 1997, 1562 and Khor, Proceedings of the American Society of Clinical Oncology (Abstract), 1997, 847).

Bispecific antibodies comprising a region directed against EpCAM and a region directed against CD3 have also been described. The authors of Möller & Reisfeld 1991 Cancer Immunol. Immunother. 33:210-216 describe the construction of two different bispecific antibodies by fusing a hybridoma producing monoclonal antibody against EpCAM with either of the two hybridomas OKT3 and 9.3. Furthermore, Kroesen, Cancer Research, 1995, 55:4409-4415 describe a

quadroma bispecific monoclonal antibodies against CD3 (BIS-1) and EpCAM.

Other examples of bispecific antibodies against EpCAM comprise the bispecific antibody, BiUII, (anti-CD3 (rat IgG2b) x anti-EpCAM (mouse IgG2a)) a complete Ig molecule which also binds and activates Fc-receptor positive accessory cells (like monocytes/macrophages, NK cells and dendritic cells) through its Fc-region (Zeidler, J. Immunol., 1999, 163:1247-1252) and an anti-EpCAMxanti-CD3 bispecific antibody in the arrangement  $V_{L17-1A}-V_{H17-1A}-V_{Hanti-CD3}-V_{Lanti-CD3}$  (Mack, Proc. Natl. Acad. Sci., 1995, 92:7021-7025).

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In addition, other formats of antibody constructs comprising EpCAM have been described; e.g. a bispecific diabody having the structure  $V_{H anti-CD3}-V_{L anti-EpCAM}-V_{H anti-EpCAM}-V_{L anti-CD3}$  (Helfrich, Int. J. Cancer, 1998, 76:232-239) and a trispecific antibody having two different tumour antigen specificities (two antigen binding regions which bind two different antigens on a tumour cell) and which may have a further specificity for an antigen localized on an effector cell (DE 195 31 348).

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There exist various descriptions in the prior art of using phage display technology to identify antibodies or fragments thereof, which specifically bind to the human EpCAM antigen (De Kruif JMB, 1995, 248:97-105, WO 99/25818). However, it has been extremely difficult to identify antibodies against EpCAM, which show cytotoxic activity sufficient for therapeutic applications in a bispecific format.

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It is therefore an aim of the present invention to provide a bispecific single chain molecule with a binding domain specific for EpCAM with strong cytotoxic activity mediated by target specific activation of T cells.

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Thus, the technical problem underlying the present invention was to provide means

and methods for the generation of well tolerated and convenient medicaments for the treatment and or amelioration of tumorous diseases.

5 The solution to said technical problem is achieved by providing the embodiments characterized in the claims.

Accordingly, the present invention relates to a composition, preferably a pharmaceutical composition, comprising a bispecific single chain antibody construct, whereby said construct comprises or consists of at least two binding  
10 domains, whereby one of said domains binds to human EpCAM antigen and a second domain binds to human CD3 antigen, wherein said binding domain specific for EpCAM comprises at least one CDR-H3 region comprising the amino acid sequence NXD preferably in position 102 to 104 of SEQ ID NOs: 80, 88 and 96, or preferably in position 106 to 108 of SEQ ID NOs: 84 and 92, wherein X is an  
15 aromatic amino acid.

Preferably or alternatively, the present invention relates to a composition, preferably a pharmaceutical composition, comprising a bispecific single chain antibody construct, whereby said construct comprises or consists of at least two domains, whereby one of said at least two domains specifically binds to human  
20 EpCAM antigen and a second domain binds to human CD3 antigen, wherein said binding domain specific for EpCAM comprises at least one CDR-H3 region of least 9 amino acid residues and wherein said binding domain specific for EpCAM has a  $K_D$  value of more than  $5 \times 10^{-9}$  M.

25 In accordance with this invention, the term "pharmaceutical composition" relates to a composition for administration to a patient, preferably a human patient. In a preferred embodiment, the pharmaceutical composition comprises a composition for parenteral, transdermal, intraluminal, intra-arterial, intrathecal or intravenous administration or for direct injection into the tumor. It is in particular envisaged that  
30 said pharmaceutical composition is administered to a patient via infusion or injection. Administration of the suitable compositions may be effected by different

ways, e.g., by intravenous, subcutaneous, intraperitoneal, intramuscular, topical or intradermal administration. The pharmaceutical composition of the present invention may further comprise a pharmaceutically acceptable carrier. Examples of suitable pharmaceutical carriers are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions, etc. Compositions comprising such carriers can be formulated by well known conventional methods. These pharmaceutical compositions can be administered to the subject at a suitable dose. The dosage regimen will be determined by the attending physician and clinical factors. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. A preferred dosage for administration might be in the range of 0.24  $\mu$ g to 48 mg, preferably 0.24  $\mu$ g to 24 mg, more preferably 0.24  $\mu$ g to 2.4 mg, even more preferably 0.24  $\mu$ g to 1.2 mg and most preferably 0.24  $\mu$ g to 240  $\mu$ g units per kilogram of body weight per day. Particularly preferred dosages are recited herein below. Progress can be monitored by periodic assessment. Dosages will vary but a preferred dosage for intravenous administration of DNA is from approximately  $10^6$  to  $10^{12}$  copies of the DNA molecule. The compositions of the invention may be administered locally or systematically. Administration will generally be parenteral, e.g., intravenous; DNA may also be administered directly to the target site, e.g., by biolistic delivery to an internal or external target site or by catheter to a site in an artery. In an preferred embodiment, the pharmaceutical composition is administered subcutaneously and in an even more preferred embodiment intravenously. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated

Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishes, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. In addition, the pharmaceutical composition of the present invention might comprise proteinaceous carriers, like, e.g., serum albumine or immunoglobuline, preferably of human origin. It is envisaged that the pharmaceutical composition of the invention might comprise, in addition to the proteinaceous bispecific single chain antibody constructs or nucleic acid molecules or vectors encoding the same (as described in this invention), further biologically active agents, depending on the intended use of the pharmaceutical composition. Such agents might be drugs acting on the gastro-intestinal system, drugs acting as cytostatica, drugs preventing hyperurikemia, agents such as T-cell co-stimulatory molecules or cytokines, drugs inhibiting immune reactions (e.g. corticosteroids) and/or drugs acting on the circulatory system, e.g. on the blood pressure, known in the art.

Possible indications for administration of the composition(s) of the invention are tumorous diseases especially epithelial cancers/carcinomas such as breast cancer, colon cancer, prostate cancer, head and neck cancer, skin cancer, cancers of the genito-urinary tract, e.g. ovarian cancer, endometrial cancer, cervix cancer and kidney cancer, lung cancer, gastric cancer, cancer of the small intestine, liver cancer, pancreas cancer, gall bladder cancer, cancers of the bile duct, esophagus cancer, cancer of the salivatory glands and cancer of the thyroid gland. The administration of the composition(s) of the invention is especially indicated for minimal residual disease preferably early solid tumor, advanced solid tumor or metastatic solid tumor, which is characterized by the local and non-local reoccurrence of the tumor caused by the survival of single cells.

The invention further envisages the co-administration protocols with other compounds, e.g. bispecific antibody constructs, targeted toxins or other compounds, which act via T cells. The clinical regimen for co-administration of the inventive compound(s) may encompass co-administration at the same time, before or after the administration of the other component.

A possible approach to demonstrate the efficacy/activity of the inventive constructs is an in vivo model like mouse. Suitable models may be transgenic and chimeric mouse models. Mouse models expressing human CD3 and human EpCAM, a chimeric mouse model expressing murine CD3 and into which tumour cells expressing human EpCAM can be transfected and chimeric mouse models comprising nude mice into which human tumours expressing EpCAM can be transplanted or tumour cells expressing human EpCAM can be injected and, additionally, human PBMCs are injected. The term "bispecific single chain antibody construct" relates to a construct comprising two antibody derived binding domains.

One of said binding domains consists of variable regions (or parts thereof) of an antibody, antibody fragment or derivative thereof, capable of specifically binding to/interacting with human EpCAM antigen (target molecule 1). The second binding domain consists of variable regions (or parts thereof) of an antibody, antibody fragment or derivative thereof, capable of specifically binding to/interacting with human CD3 antigen (target molecule 2). As will be detailed below, a part of a variable region may be at least one CDR ("Complementary determining region"), most preferably at least the CDR3 region. Said two domains/regions in the single chain antibody construct are preferably covalently connected to one another as a single chain. This connection can be effected either directly (domain 1 [specific for the CD3 antigen] – domain 2 [specific for the EpCAM antigen] or domain 1 [specific for the EpCAM antigen] – domain 2 [specific for the CD3 antigen]) or through an additional polypeptide linker sequence (domain1 – linker sequence – domain2). In the event that a linker is used, this linker is preferably of a length and sequence sufficient to ensure that each of the first and second domains can, independently from one another, retain their differential binding specificities. Most preferably and as documented in the appended examples, the "bispecific single chain antibody construct" to be employed in the pharmaceutical composition of the invention is a bispecific single chain Fv (scFv). Bispecific single chain molecules are known in the art and are described in WO 99/54440, Mack, J. Immunol. (1997), 158, 3965-3970, Mack, PNAS, (1995), 92, 7021-7025, Kufer, Cancer Immunol. Immunother., (1997), 45, 193-197, Löffler, Blood, (2000), 95, 6, 2098-2103 and Brühl, J.

Immunol., (2001), 166, 2420-2426. A particularly preferred molecular format of the invention provides a polypeptide construct wherein the antibody-derived region comprises one  $V_H$  and one  $V_L$  region. The intramolecular orientation of the  $V_H$ -domain and the  $V_L$ -domain, which are linked to each other by a linker-domain, in the scFv format is not decisive for the recited bispecific single chain constructs.

Thus, scFvs with both possible arrangements ( $V_H$ -domain – linker domain –  $V_L$ -domain;  $V_L$ -domain – linker domain –  $V_H$ -domain) are particular embodiments of the recited bispecific single chain construct.

The antibody construct may also comprise additional domains, e.g. for the isolation and/or preparation of recombinantly produced constructs.

A corresponding format for a bispecific single chain antibody construct is described in the appended example 1.

The term "single-chain" as used in accordance with the present invention means that said first and second domain of the bispecific single chain construct are covalently linked, preferably in the form of a co-linear amino acid sequence encodable by a single nucleic acid molecule.

The term "binding to/interacting with" as used in the context with the present invention defines a binding/interaction of at least two "antigen-interaction-sites" with each other. The term "antigen-interaction-site" defines, in accordance with the present invention, a motif of a polypeptide which shows the capacity of specific interaction with a specific antigen or a specific group of antigens. Said binding/interaction is also understood to define a "specific recognition". The term "specifically recognizing" means in accordance with this invention that the antibody molecule is capable of specifically interacting with and/or binding to at least two amino acids of each of the human target molecule as defined herein. Said term relates to the specificity of the antibody molecule, i.e. to its ability to discriminate between the specific regions of the human target molecule as defined herein. The specific interaction of the antigen-interaction-site with its specific antigen may result in an initiation of a signal, e.g. due to the induction of a change of the conformation of the antigen, an oligomerization of the antigen, etc. Further, said binding may be

exemplified by the specificity of a "key-lock-principle". Thus, specific motifs in the amino acid sequence of the antigen-interaction-site and the antigen bind to each other as a result of their primary, secondary or tertiary structure as well as the result of secondary modifications of said structure. The specific interaction of the antigen-interaction-site with its specific antigen may result as well in a simple binding of said site to the antigen.

The term "specific interaction" as used in accordance with the present invention means that the bispecific single chain construct does not or essentially does not cross-react with (poly)peptides of similar structures. Cross-reactivity of a panel of bispecific single chain construct under investigation may be tested, for example, by assessing binding of said panel of bispecific single chain construct under conventional conditions (see, e.g., Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1988 and Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1999) to the (poly)peptide of interest as well as to a number of more or less (structurally and/or functionally) closely related (poly)peptides. Only those antibodies that bind to the (poly)peptide/protein of interest but do not or do not essentially bind to any of the other (poly)peptides are considered specific for the (poly)peptide/protein of interest. Examples for the specific interaction of an antigen-interaction-site with a specific antigen comprise the specificity of a ligand for its receptor. Said definition particularly comprises the interaction of ligands which induce a signal upon binding to its specific receptor. Examples for corresponding ligands comprise cytokines which interact/bind with/to its specific cytokine-receptors. Also particularly comprised by said definition is the binding of an antigen-interaction-site to antigens like antigens of the selectin family, integrins and of the family of growth factors like EGF. An other example for said interaction, which is also particularly comprised by said definition, is the interaction of an antigenic determinant (epitope) with the antigenic binding site of an antibody.

The term "binding to/interacting with" may also relate to a conformational epitope, a structural epitope or a discontinuous epitope consisting of two regions of the human target molecules or parts thereof. In context of this invention, a



conformational epitope is defined by two or more discrete amino acid sequences separated in the primary sequence which come together on the surface of the molecule when the polypeptide folds to the native protein (Sela, (1969) Science 166, 1365 and Laver, (1990) Cell 61, 553-6).

- 5 The term "discontinuous epitope" means in context of the invention non-linear epitopes that are assembled from residues from distant portions of the polypeptide chain. These residues come together on the surface of the molecule when the polypeptide chain folds into a three-dimensional structure to constitute a conformational/structural epitope.
- 10 The constructs of the present invention are also envisaged to specifically bind to/interact with a conformational/structural epitope(s) composed of and/or comprising the two regions of the human CD3 complex described herein or parts thereof as disclosed herein below.

Accordingly, specificity can be determined experimentally by methods known in the art and methods as disclosed and described herein. Such methods comprise, but  
15 are not limited to Western blots, ELISA-, RIA-, ECL-, IRMA-, EIA-tests and peptide scans.

The term "antibody fragment or derivative thereof" relates to single chain antibodies, or fragments thereof, synthetic antibodies, antibody fragments, such as  
20 Fab, a F(ab<sub>2</sub>)', Fv or scFv fragments etc., or a chemically modified derivative of any of these. Antibodies to be employed in accordance with the invention or their corresponding immunoglobulin chain(s) can be further modified using conventional techniques known in the art, for example, by using amino acid deletion(s), insertion(s), substitution(s), addition(s), and/or recombination(s) and/or any other  
25 modification(s) (e.g. posttranslational and chemical modifications, such as glycosylation and phosphorylation) known in the art either alone or in combination. Methods for introducing such modifications in the DNA sequence underlying the amino acid sequence of an immunoglobulin chain are well known to the person skilled in the art; see, e.g., Sambrook (1989), loc. cit.

- 30 The term "(poly)peptide" as used herein describes a group of molecules which comprise the group of peptides, as well as the group of polypeptides. The group of

peptides is consisting of molecules with up to 30 amino acids, the group of polypeptides is consisting of molecules with more than 30 amino acids.

The term "antibody fragment or derivative thereof" particularly relates to (poly)peptide constructs comprising at least one CDR.

- 5 Fragments or derivatives of the recited antibody molecules define (poly)peptides which are parts of the above antibody molecules and/or which are modified by chemical/biochemical or molecular biological methods. Corresponding methods are known in the art and described inter alia in laboratory manuals (see Sambrook et al.; Molecular Cloning: A Laboratory Manual; Cold Spring Harbor Laboratory Press, 10 2nd edition 1989 and 3rd edition 2001; Gerhardt et al.; Methods for General and Molecular Bacteriology; ASM Press, 1994; Lefkovits; Immunology Methods Manual: The Comprehensive Sourcebook of Techniques; Academic Press, 1997; Golemis; Protein-Protein Interactions: A Molecular Cloning Manual; Cold Spring Harbor Laboratory Press, 2002).
- 15 Bispecific antibodies that specifically recognize the EpCAM antigen and the CD3 antigen are described in the prior art, e.g., in Mack (Proc. Natl. Acad. Sci., 1995, 92:7021-7025).
- 20 As mentioned above, the said variable domains comprised in the herein described bispecific single chain constructs are connected by additional linker sequences. The term "peptide linker" defines in accordance with the present invention an amino acid sequence by which the amino acid sequences of the first domain and the second domain of the defined construct are linked with each other. An essential 25 technical feature of such peptide linker is that said peptide linker does not comprise any polymerization activity. A particularly preferred peptide linker is characterized by the amino acid sequence Gly-Gly-Gly-Gly-Ser, i.e. (Gly)<sub>4</sub>Ser, or polymers thereof, i.e. ((Gly)<sub>4</sub>Ser)<sub>x</sub>. The characteristics of said peptide linker, which comprise the absence of the promotion of secondary structures are known in the art and 30 described e.g. in Dall'Acqua et al. (Biochem. (1998) 37, 9266-9273), Cheadle et al. (Mol Immunol (1992) 29, 21-30) and Raag and Whitlow (FASEB (1995) 9(1), 73-

- 80). Also particularly preferred are peptide linkers which comprise less amino acid residues. An envisaged peptide linker with less than 5 amino acids can comprise 4, 3, 2 or one amino acids. A particularly preferred "single" amino acid in context of said "peptide linker" is Gly. Accordingly, said peptide linker may consist of the single amino acid Gly. Furthermore, peptide linkers which also do not promote any secondary structures are preferred. The linkage of said domains to each other can be provided by, e.g. genetic engineering, as described in the examples. Methods for preparing fused and operatively linked bispecific single chain constructs and expressing them in mammalian cells or bacteria are well-known in the art (e.g. WO 99/54440, Ausubel, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y. 1989 and 1994 or Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001).
- 15 The bispecific single chain antibody constructs described herein above and below may be humanized or deimmunized antibody constructs. Methods for the humanization and/or deimmunization of (poly))peptides and, in particular, antibody constructs are known to the person skilled in the art.
- 20 Here it was surprisingly found that domains with specificity for the EpCAM antigen, comprising at least one CDR-H3 region comprising the amino acid sequence NXD (asparagine-X-aspartic acid) preferably in position 102 to 104 of SEQ ID NOs: 80, 88 and 96, or in position 106 to 108 of SEQ ID NOs: 84 and 92, wherein X is an aromatic amino acid are particularly useful in the specific format of a bispecific
- 25 single chain antibody construct. These bispecific single chain antibody constructs are particularly useful as pharmaceutical compositions since these constructs are advantageous over constructs which do not comprise said amino acids.
- Furthermore, it was surprisingly found that domains with specificity for the EpCAM antigen, comprising at least one CDR-H3 region of at least 9 amino acid residues and having a  $K_D$  value of more than  $5 \times 10^{-9}$  M are particularly useful in the specific
- 30 format of a bispecific single chain antibody construct. These bispecific single chain

antibody construct are particularly useful as pharmaceutical compositions since these constructs are advantageous over constructs of less than 9 amino acid residues and wherein said binding domain specific for EpCAM has a  $K_D$  value of less than  $5 \times 10^{-9}$  M.

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The prior art constructs are characterized by less advantageous  $EC_{50}$  values and/or less efficient or complete purifications as shown in the appended examples. It was in particular surprising that the domain of the single chain constructs with specificity for the CD3 antigen to be employed in accordance with the invention are highly bioactive in N- as well as C-terminal position, wherein in particular arrangements in  $V_{H(anti-CD3)}-V_{L(anti-CD3)}$  are preferred. The constructs to be employed in the pharmaceutical composition of the invention are characterized by advantageous production and purification properties as well as by their high bioactivity, i.e. their desired cytotoxic activity. In particular, when the cytotoxic activity of the constructs of the invention were compared with cytotoxic activity of conventional M79xanti-CD3 and HD70xanti-CD3 constructs, the constructs of the invention showed clearly higher bioactivity (Figure 11B). The corresponding high bioactivity is reflected by low to very low  $EC_{50}$  values as determined in cytotoxicity tests. The lower the  $EC_{50}$  value of the molecule is, the higher cytotoxicity, i.e. the effectivity in the cell lysis, of the construct is higher. On the other hand, the higher the  $EC_{50}$  value, the less effective the molecule is in inducing cell lysis. The term " $EC_{50}$ " corresponds, in context of this invention, to  $EC_{50}$  values as determined according to the methods known in the art and as illustrated in the appended examples: A standard dose-response curve is defined by four parameters: the baseline response (Bottom), the maximum response (Top), the slope, and the drug concentration that provokes a response halfway between baseline and maximum ( $EC_{50}$ ).  $EC_{50}$  is defined as the concentration of a drug or molecule that provokes a response half way between the baseline (Bottom) and maximum response (Top). A lower  $K_D$  value of the constructs of the invention depicts higher binding affinity. E.g. a low  $K_D$  of  $10^{-9}$  M shows high binding affinity of the binding construct. On the other hand a high  $K_D$  value of e.g.  $10^{-6}$  M relates to lower binding affinity of the binding

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domain of the construct.

The percentage of cell lysis (i.e. cytotoxic activity) may be determined by, inter alia, release assays disclosed herein above, for example,  $^{51}\text{Cr}$  release assays, LDH-release assays and the like. Most preferably, in context of this invention  
5 fluorochrome release assays is employed as illustrated in the appended examples. Here, strong cytotoxic activity against EpCAM-positive cells (see CHO-EpCAM cells in appended example 3) of the bispecific single chain constructs described herein relates to a molecule comprising  $\text{EC}_{50}$  values preferably  $\leq 500$  pg/ml, more preferably  $\leq 400$  pg/ml, even more preferably  $\leq 300$  pg/ml, even more preferably  $\leq$   
10 250 pg/ml, most preferably  $\leq 200$  pg/ml  $\leq 100$  pg/ml,  $\leq 50$  pg/ml.

The bispecific constructs comprised in the pharmaceutical compositions of the present invention show a surprisingly high cytotoxic activity (preferably in the range of about 10 pg/ml to 170 pg/ml) compared to the prior art M79xanti-CD3 construct  
15 (VL<sub>17-1A</sub>- VH<sub>17-1A</sub>- VH<sub>CD3</sub>- VL<sub>CD3</sub>; 8628 pg/ml). A skilled person is aware that  $\text{EC}_{50}$  values may vary depending to the bioactivity assay. Factors affecting  $\text{EC}_{50}$  value may comprise type of effector cells, activity of effector cells, type of target cells, E:T ratio, incubation time, incubation temperature and other external circumstances. Different  $\text{EC}_{50}$  values of same constructs in different experiments may be  
20 compared with the  $\text{EC}_{50}$  values of controls. A construct having high cytotoxic activity according to the invention has at least 2.5 time lower  $\text{EC}_{50}$  value than the control (at least 2.5 times higher cytotoxicity than the control), preferably at least three times lower  $\text{EC}_{50}$  value and more preferably at least five times lower  $\text{EC}_{50}$  value.

25 Furthermore, the constructs of the invention bind EpCAM with a surprisingly high affinity measured by surface plasmon resonance (BIAcore®). The prior art EpCAM and CD3 binding construct M79xanti-CD3 has a  $K_D$  of  $4 \times 10^{-6}$  M and the constructs of the invention a  $K_D$  of  $2,3 \times 10^{-7} - 2,5 \times 10^{-7}$  M.

30 Preferably, the X in said NXD motif is W (tryptophan) or Y (tyrosine).

It is further envisaged that the pharmaceutical composition of the invention comprises a bispecific single chain antibody construct, wherein the CDR-H3 of the EpCAM specific domain comprises at least 9 amino acid residues, preferably at least 14 amino acids. Preferably the CDR-H3 comprises less than 18 amino acids, more preferably less than 15 amino acids. Thus, preferably the CDR-H3 comprises 9 to 17 amino acids, more preferably 9 to 15 amino acids and most preferably 10 or 14 amino acids.

Bispecific single chain antibody construct comprising a corresponding EpCAM specific domain have been surprisingly found to be advantageous in the format of the above described construct over other EpCAM specific domain known in the art. Such effect is demonstrated in appended examples 3, 4 and 5. The prior art EpCAM binding antibody M79 comprises eight amino acids in its CDR-H3 region and does not comprise the sequence NXD (Figure 11A).

The pharmaceutical composition according to the invention may also comprise constructs, wherein said binding domain specific for EpCAM has a  $K_D$  value of more than  $5 \times 10^{-9}$  M.

The pharmaceutical composition may additionally be characterized by the feature that said binding domain specific for the CD3 antigen has a  $K_D$  value of more than  $10^{-7}$  M.

The  $K_D$  value is a physical value defining the tendency of a complex to dissociate. For the binding equilibrium  $A+B \leftrightarrow AB$ , the dissociation constant is given as the ratio of the two kinetic rate constants  $k_{off}$  and  $k_{on}$ :  $[A][B] (k_{on})/[AB] (k_{off})$ . The smaller the dissociation constant the tighter A and B bind to each other. In biological systems a good, specific binder has a dissociation constant in the range of  $10^{-9}$ - $10^{-7}$  M.  $K_D$  can be measured with a number of methods known to the person skilled in the art, e.g. surface plasmon resonance (SPR, e.g. with BIAcore®), analytical ultracentrifugation, isothermal titration calorimetry, fluorescence anisotropy, fluorescence spectroscopy or by radiolabeled ligand binding assays.

The  $K_D$ s of the constructs of the invention have been measured using the surface

plasmon resonance (SPR) spectroscopy. The ligand is injected over the immobilized antigen chip surface and the change in optical density on the chip surface upon binding is measured. The change in optical density, monitored by a change in reflection angle, correlates directly to the amount of ligand binding to the chip surface - the biophysical phenomenon used is called surface plasmon resonance.

One of the interaction partners has to be immobilized on the surface of the sensor chip of the apparatus based on surface plasmon resonance (e.g. BIAcore®). The kinetics of association and dissociation of ligand with the immobilized antigen on the chip surface are observed in real time. The binding curves are fitted for kinetic rate constants  $k_{on}$  and  $k_{off}$ , resulting in an apparent equilibrium dissociation constant ( $K_D$ ).

It is particularly preferred, that said binding domain specific for EpCAM has a  $K_D$  value in a range between  $1 \times 10^{-7}$  and  $5 \times 10^{-9}$  M and said binding domain specific for CD3 has a  $K_D$  value in a range between  $1 \times 10^{-6}$  and  $5 \times 10^{-9}$  M.

In a particularly preferred embodiment, the pharmaceutical composition may additionally be characterized by the feature that said binding domain specific for the CD3 antigen has a  $K_D$  value of  $> (more\ than) 1 \times 10^{-7}$  M.

The constructs of the invention have the advantage that they may be used a number of times for killing tumour cells since the EpCAM binding part has an affinity with a  $K_D$  value of more than  $5 \times 10^{-9}$  M. If the affinity of a bispecific construct for binding an EpCAM-expressing tumour cell is too high, the construct binds one EpCAM expressing tumour cell and remains on its surface even when it has been killed and cannot continue to another tumour cell to be killed. A further advantage of the construct of the invention is that the binding domain specific for EpCAM binds with a high affinity (corresponds to lower  $K_D$  value), thus leading the circulating T-cells to the tumour cells marked with the bispecific construct. Therefore, the  $K_D$  of the binding domain specific for EpCAM of the bispecific construct is preferably in the range of  $10^{-7}$ - $5 \times 10^{-9}$  M and the  $K_D$  of the binding

domain specific for CD3 is preferably in the range of  $10^{-6}$  -  $5 \times 10^{-9}$  M. In a preferred embodiment, the KD value of the EpCAM binding domain is lower than the KD value of the CD3 binding domain corresponding to a higher affinity of the EpCAM binding domain compared to the CD3 binding domain.

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Further it is envisaged that the pharmaceutical composition of the invention comprises a bispecific single chain antibody construct, wherein the CDR-H3 of the EpCAM specific domain comprises at least 9 amino acids, preferably at least 14 amino acids. Preferably the CDR-H3 comprises less than 18 amino acids, more  
10 preferably less than 15 amino acids. Thus, preferably the CDR-H3 comprises 9 to 17 amino acids, more preferably 9 to 15 amino acids and most preferably 10 or 14 amino acids.

In a preferred embodiment of the pharmaceutical composition of the invention the  
15  $V_H$  chain of the domain specific for human EpCAM antigen is selected from the group consisting of:

- (a) an amino acid sequence as shown in any of SEQ ID NO: 80, SEQ ID NO: 84, SEQ ID NO: 88, SEQ ID NO: 92 and SEQ ID NO: 96;
- (b) an amino acid sequence encoded by a nucleic acid sequence as shown in  
20 SEQ ID NO: 79, SEQ ID NO: 83, SEQ ID NO: 87, SEQ ID NO: 91 and SEQ ID NO: 95 ;
- (c) an amino acid sequence encoded by a nucleic acid sequence hybridizing with the complementary strand of a nucleic acid sequence as defined in (b) under stringent hybridization conditions;
- 25 (d) an amino acid sequence encoded by a nucleic acid sequence which is degenerate as a result of the genetic code to a nucleotide sequence of any one of (b) and (c).

The term "hybridizing" as used herein refers to polynucleotides/nucleic acid sequences which are capable of hybridizing to the polynucleotides encoding  
30 bispecific single chain constructs as defined herein or parts thereof. Therefore, said polynucleotides may be useful as probes in Northern or Southern Blot analysis of



RNA or DNA preparations, respectively, or can be used as oligonucleotide primers in PCR analysis dependent on their respective size. Preferably, said hybridizing polynucleotides comprise at least 10, more preferably at least 15 nucleotides in length while a hybridizing polynucleotide of the present invention to be used as a probe preferably comprises at least 100, more preferably at least 200, or most preferably at least 500 nucleotides in length.

It is well known in the art how to perform hybridization experiments with nucleic acid molecules, i.e. the person skilled in the art knows what hybridization conditions s/he has to use in accordance with the present invention. Such hybridization conditions are referred to in standard text books such as Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory (2001) N.Y. Preferred in accordance with the present inventions are polynucleotides which are capable of hybridizing to the polynucleotides of the invention or parts thereof, under stringent hybridization conditions.

"Stringent hybridization conditions" refer, e.g. to an overnight incubation at 42°C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM sodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1 x SSC at about 65°C. Also contemplated are nucleic acid molecules that hybridize to the polynucleotides of the invention at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37°C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M NaH<sub>2</sub>PO<sub>4</sub>; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 µg/ml salmon sperm blocking DNA; followed by washes at 50°C with 1 X SSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC). It is of note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to

suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

The recited nucleic acid molecules may be, e.g., DNA, cDNA, RNA or synthetically produced DNA or RNA or a recombinantly produced chimeric nucleic acid molecule comprising any of those polynucleotides either alone or in combination.

10 Preferably said pharmaceutical composition of the invention may comprise a bispecific single chain construct, wherein the  $V_L$  chain domains specific for human EpCAM antigen is selected from the group consisting of:

- (a) an amino acid sequence as shown in any of SEQ ID NO: 82, SEQ ID NO: 86, SEQ ID NO: 90, SEQ ID NO: 94 and SEQ ID NO: 98;
- 15 (b) an amino acid sequence encoded by a nucleic acid sequence as shown in SEQ ID NO: 81, SEQ ID NO: 85, SEQ ID NO: 89, SEQ ID NO: 93 and SEQ ID NO: 97 ;
- (c) an amino acid sequence encoded by a nucleic acid sequence hybridizing with the complementary strand of a nucleic acid sequence as defined in (b) under stringent hybridization conditions;
- 20 (d) an amino acid sequence encoded by a nucleic acid sequence which is degenerate as a result of the genetic code to a nucleotide sequence of any one of (b) and (c).

25 In a preferred embodiment of the pharmaceutical composition of this invention, the  $V_H$  and  $V_L$  regions of said human CD3 specific domain are derived from an CD3 specific antibody selected from the group consisting of X35-3, VIT3, BMA030 (BW264/56), CLB-T3/3, CRIS7, YTH12.5, F111-409, CLB-T3.4.2, TR-66, WT32, SPv-T3b, 11D8, XIII-141, XIII-46, XIII-87, 12F6, T3/RW2-8C8, T3/RW2-4B6, 30 OKT3D, M-T301, SMC2, WT31 and F101.01. These CD3-specific antibodies are well known in the art and, inter alia, described in Tunnacliffe (1989), Int. Immunol.

1, 546-550. In a more preferred embodiment, said  $V_H$  and  $V_L$  regions of said CD3 specific domain are derived from OKT-3 (as defined and described above). Even more preferred (and as illustrated in the appended examples) said  $V_H$  and  $V_L$  regions are or are derived from an antibody/antibody derivative with specificity for the CD3 molecule described by Traunecker (1991), EMBO J. 10, 3655-3659. In accordance with this invention, said  $V_H$  and  $V_L$  regions are derived from antibodies/antibody derivatives and the like which are capable of specifically recognizing the human CD3- $\epsilon$  chain in the context of other TCR subunits, e.g. in mouse cells transgenic for human CD3- $\epsilon$  chain. These transgenic mouse cells express human CD3- $\epsilon$  chain in a native or near native conformation. Accordingly, the  $V_H$  and  $V_L$  regions derived from an CD3- $\epsilon$  chain specific antibody is most preferred in accordance with this invention and said (parental) antibodies should be capable of specifically binding epitopes reflecting the native or near native structure or a conformational epitope of human CD3 presented in context of the TCR complex. Such antibodies have been classified by Tunncliffe (1989) as "group II" antibodies. Further classifications in Tunncliffe (1989) comprise the definition of "group I" and "group III" antibodies directed against CD3. "Group I" antibodies, like UCHT1, recognize CD3- $\epsilon$  chain expressed as recombinant protein and as part of the TCR on the cell surface. Therefore, "group I" antibodies are highly specific for CD3- $\epsilon$  chain. In contrast, the herein preferred "group II antibodies" recognize CD3- $\epsilon$  chain only in the native TCR complex in association with other TCR subunits. Without being bound by theory, it is speculated in context of this invention that in "group II" antibodies, the TCR context is required for recognition of CD3- $\epsilon$  chain. CD3- $\gamma$  chain and  $\delta$  chain, being associated with  $\epsilon$  chain, are also involved in binding of "group II antibodies". All three subunits express immunoreceptor-tyrosine activation motifs (ITAMs) which can be tyrosine phosphorylated by protein tyrosine-based kinases. For this reason group II antibodies induce T cell signaling via CD3- $\epsilon$  chain,  $\gamma$  chain and  $\delta$  chain, leading to a stronger signal compared to group I antibodies selectively inducing T cell signaling via CD3- $\epsilon$  chain. Yet, since for therapeutic applications induction of a strong T cell signaling is desired, the  $V_H$  (anti-CD3) /  $V_L$  (anti-CD3)- regions (or parts thereof) to be employed in the bispecific single

chain constructs comprised in the inventive pharmaceutical composition, are preferably derived from antibodies directed against human CD3 and classified in "group II" by Tunnacliffe (1989), loc.cit.

- 5 In one embodiment the present invention relates to a pharmaceutical composition wherein said bispecific single chain antibody construct comprises an amino acid sequence selected from the group of:
- (a) an amino acid sequence as shown in any of SEQ ID NOs: 2, 4, 8, 10, 12, 14, 16, 18, 20, 30, 36, 39, 42, 44, 46, 48, 50, 52, 54, 56, 58 and 60;
  - 10 (b) an amino acid sequence encoded by a nucleic acid sequence as shown in any of SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 29, 35, 38, 41, 43, 45, 47, 49, 51, 53, 55, 57 and 59;
  - (c) an amino acid sequence encoded by a nucleic acid sequence hybridizing with the complementary strand of a nucleic acid sequence as defined in (b)
  - 15 under stringent hybridization conditions;
  - (d) an amino acid sequence encoded by a nucleic acid sequence which is degenerate as a result of the genetic code to a nucleotide sequence of any one of (b) and (c).
- 20 The present invention also provides for a pharmaceutical composition comprising a nucleic acid sequence encoding a bispecific single chain antibody construct as defined above.
- Said nucleic acid molecule may be a natural nucleic acid molecule as well as a recombinant nucleic acid molecule. The nucleic acid molecule may, therefore, be
- 25 of natural origin, synthetic or semi-synthetic. It may comprise DNA, RNA as well as PNA (peptide nucleic acid) and it may be a hybrid thereof.
- Thus, the present invention relates to a pharmaceutical composition comprising a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:
- 30 (a) a nucleotide sequence encoding the mature form of a protein comprising the amino acid sequence of the bispecific single chain antibody constructs

defined herein, preferably as given in SEQ ID Nos: 2, 4, 8, 10, 12, 14, 16, 18, 20, 30, 36, 39, 42, 44, 46, 48, 50, 52, 54, 56, 58 and 60;

- (b) a nucleotide sequence comprising or consisting of the DNA sequence as given in SEQ ID Nos: 1, 3, 7, 9, 11, 13, 15, 17, 19, 29, 35, 38, 41, 43, 45, 47, 49, 51, 53, 55, 57 and 59;
- (c) a nucleotide sequence hybridizing with the complementary strand of a nucleotide sequence as defined in (b) under stringent hybridization conditions;
- (d) a nucleotide sequence encoding a protein derived from the protein encoded by a nucleotide sequence of (a) or (b) by way of substitution, deletion and/or addition of one or several amino acids of the amino acid sequence encoded by the nucleotide sequence of (a) or (b);
- (e) a nucleotide sequence encoding a protein having an amino acid sequence at least 60 % identical to the amino acid sequence encoded by the nucleotide sequence of (a) or (b);
- (f) a nucleotide sequence which is degenerate as a result of the genetic code to a nucleotide sequence of any one of (a) to (e);

The term "mature form of the protein" defines in context with the present invention a protein translated from its corresponding mRNA and optional subsequently modified.

The term "hybridizing" has been defined in the context of the present invention herein above.

It is evident to the person skilled in the art that regulatory sequences may be added to the nucleic acid molecule comprised in the pharmaceutical composition of the invention. For example, promoters, transcriptional enhancers and/or sequences which allow for induced expression of the polynucleotide of the invention may be employed. A suitable inducible system is for example tetracycline-regulated gene expression as described, e.g., by Gossen and Bujard (Proc. Natl. Acad. Sci. USA 89 (1992), 5547-5551) and Gossen et al. (Trends Biotech. 12 (1994), 58-62), or a dexamethasone-inducible gene expression system as described, e.g. by Crook (1989) EMBO J. 8, 513-519 .

Furthermore, it is envisaged for further purposes that nucleic acid molecules may contain, for example, thioester bonds and/or nucleotide analogues. Said modifications may be useful for the stabilization of the nucleic acid molecule against endo- and/or exonucleases in the cell. Said nucleic acid molecules may be transcribed by an appropriate vector containing a chimeric gene which allows for the transcription of said nucleic acid molecule in the cell. In this respect, it is also to be understood that such polynucleotide can be used for "gene targeting" or "gene therapeutic" approaches. In another embodiment said nucleic acid molecules are labeled. Methods for the detection of nucleic acids are well known in the art, e.g., Southern and Northern blotting, PCR or primer extension. This embodiment may be useful for screening methods for verifying successful introduction of the nucleic acid molecules described above during gene therapy approaches. Said nucleic acid molecule(s) may be a recombinantly produced chimeric nucleic acid molecule comprising any of the aforementioned nucleic acid molecules either alone or in combination. Preferably, the nucleic acid molecule is part of a vector.

The present invention therefore also relates to a pharmaceutical composition comprising a vector comprising the nucleic acid molecule described in the present invention.

Many suitable vectors are known to those skilled in molecular biology, the choice of which would depend on the function desired and include plasmids, cosmids, viruses, bacteriophages and other vectors used conventionally in genetic engineering. Methods which are well known to those skilled in the art can be used to construct various plasmids and vectors; see, for example, the techniques described in Sambrook et al. (loc cit.) and Ausubel, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y. (1989), (1994). Alternatively, the polynucleotides and vectors of the invention can be reconstituted into liposomes for delivery to target cells. As discussed in further details below, a cloning vector was used to isolate individual sequences of DNA. Relevant sequences can be transferred into expression vectors where expression of a

particular polypeptide is required. Typical cloning vectors include pBluescript SK, pGEM, pUC9, pBR322 and pGBT9. Typical expression vectors include pTRE, pCAL-n-EK, pESP-1, pOP13CAT.

Preferably said vector comprises a nucleic acid sequence which is a regulatory  
5 sequence operably linked to said nucleic acid sequence encoding a bispecific single chain antibody constructs defined herein.

Such regulatory sequences (control elements) are known to the artisan and may include a promoter, a splice cassette, translation initiation codon, translation and insertion site for introducing an insert into the vector. Preferably, said nucleic acid  
10 molecule is operatively linked to said expression control sequences allowing expression in eukaryotic or prokaryotic cells.

It is envisaged that said vector is an expression vector comprising the nucleic acid molecule encoding a bispecific single chain antibody constructs defined herein.

The term "regulatory sequence" refers to DNA sequences which are necessary to  
15 effect the expression of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism. In prokaryotes, control sequences generally include promoters, ribosomal binding sites, and terminators. In eukaryotes generally control sequences include promoters, terminators and, in some instances, enhancers, transactivators or transcription  
20 factors. The term "control sequence" is intended to include, at a minimum, all components the presence of which are necessary for expression, and may also include additional advantageous components.

The term "operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner.  
25 A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences. In case the control sequence is a promoter, it is obvious for a skilled person that double-stranded nucleic acid is preferably used.

Thus, the recited vector is preferably an expression vector. An "expression vector"  
30 is a construct that can be used to transform a selected host and provides for expression of a coding sequence in the selected host. Expression vectors can for

instance be cloning vectors, binary vectors or integrating vectors. Expression comprises transcription of the nucleic acid molecule preferably into a translatable mRNA. Regulatory elements ensuring expression in prokaryotes and/or eukaryotic cells are well known to those skilled in the art. In the case of eukaryotic cells they  
5 comprise normally promoters ensuring initiation of transcription and optionally poly-A signals ensuring termination of transcription and stabilization of the transcript. Possible regulatory elements permitting expression in prokaryotic host cells comprise, e.g., the *P<sub>L</sub>*, *lac*, *trp* or *tac* promoter in *E. coli*, and examples of regulatory elements permitting expression in eukaryotic host cells are the *AOX1* or  
10 *GAL1* promoter in yeast or the CMV-, SV40-, RSV-promoter (Rous sarcoma virus), CMV-enhancer, SV40-enhancer or a globin intron in mammalian and other animal cells.

Beside elements which are responsible for the initiation of transcription such regulatory elements may also comprise transcription termination signals, such as  
15 the SV40-poly-A site or the tk-poly-A site, downstream of the polynucleotide. Furthermore, depending on the expression system used leader sequences capable of directing the polypeptide to a cellular compartment or secreting it into the medium may be added to the coding sequence of the recited nucleic acid sequence and are well known in the art; see also, e.g., the appended examples.  
20 The leader sequence(s) is (are) assembled in appropriate phase with translation, initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein, or a portion thereof, into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired  
25 characteristics, e.g., stabilization or simplified purification of expressed recombinant product; see supra. In this context, suitable expression vectors are known in the art such as Okayama-Berg cDNA expression vector pcDV1 (Pharmacia), pEF-Neo, pCDM8, pRc/CMV, pcDNA1, pcDNA3 (In-vitrogene), pEF-DHFR and pEF-ADA, (Raum et al. Cancer Immunol Immunother (2001) 50(3), 141-  
30 150) or pSPORT1 (GIBCO BRL).

Preferably, the expression control sequences will be eukaryotic promoter systems



in vectors capable of transforming or transfecting eukaryotic host cells, but control sequences for prokaryotic hosts may also be used. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and as desired, the collection and purification of the polypeptide of the invention may follow; see, e.g., the appended examples.

An alternative expression system which could be used to express a cell cycle interacting protein is an insect system. In one such system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. The coding sequence of a recited nucleic acid molecule may be cloned into a nonessential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of said coding sequence will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein coat. The recombinant viruses are then used to infect *S. frugiperda* cells or *Trichoplusia* larvae in which the protein of the invention is expressed (Smith, J. Virol. 46 (1983), 584; Engelhard, Proc. Nat. Acad. Sci. USA 91 (1994), 3224-3227).

Additional regulatory elements may include transcriptional as well as translational enhancers. Advantageously, the above-described vectors of the invention comprises a selectable and/or scorable marker.

Selectable marker genes useful for the selection of transformed cells and, e.g., plant tissue and plants are well known to those skilled in the art and comprise, for example, antimetabolite resistance as the basis of selection for dhfr, which confers resistance to methotrexate (Reiss, Plant Physiol. (Life Sci. Adv.) 13 (1994), 143-149); npt, which confers resistance to the aminoglycosides neomycin, kanamycin and paromycin (Herrera-Estrella, EMBO J. 2 (1983), 987-995) and hygromycin (Marsh, Gene 32 (1984), 481-485). Additional selectable genes have been described, namely trpB, which allows cells to utilize indole in place of tryptophan; hisD, which allows cells to utilize histinol in place of histidine (Hartman, Proc. Natl. Acad. Sci. USA 85 (1988), 8047); mannose-6-phosphate isomerase which allows cells to utilize mannose (WO 94/20627) and

ODC (ornithine decarboxylase) which confers resistance to the ornithine decarboxylase inhibitor, 2-(difluoromethyl)-DL-ornithine, DFMO (McConlogue, 1987, In: Current Communications in Molecular Biology, Cold Spring Harbor Laboratory ed.) or deaminase from *Aspergillus terreus* which confers resistance to

5 Blasticidin S (Tamura, Biosci. Biotechnol. Biochem. 59 (1995), 2336-2338).

Useful scorable markers are also known to those skilled in the art and are commercially available. Advantageously, said marker is a gene encoding luciferase (Giacomin, Pl. Sci. 116 (1996), 59-72; Scikantha, J. Bact. 178 (1996), 121), green fluorescent protein (Gerdes, FEBS Lett. 389 (1996), 44-47) or  $\beta$ -glucuronidase

10 (Jefferson, EMBO J. 6 (1987), 3901-3907). This embodiment is particularly useful for simple and rapid screening of cells, tissues and organisms containing a recited vector.

As described above, the recited nucleic acid molecule can be used alone or as part of a vector to express the encoded polypeptide in cells, for, e.g., gene therapy. The

15 nucleic acid molecules or vectors containing the DNA sequence(s) encoding any one of the above described bispecific single chain antibody constructs is introduced into the cells which in turn produce the polypeptide of interest. Gene therapy, which is based on introducing therapeutic genes into cells by ex-vivo or in-vivo techniques is one of the most important applications of gene transfer. Suitable

20 vectors, methods or gene-delivery systems for in-vitro or in-vivo gene therapy are described in the literature and are known to the person skilled in the art; see, e.g., Giordano, Nature Medicine 2 (1996), 534-539; Schaper, Circ. Res. 79 (1996), 911-919; Anderson, Science 256 (1992), 808-813; Verma, Nature 389 (1994), 239; Isner, Lancet 348 (1996), 370-374; Muhlhauser, Circ. Res. 77 (1995), 1077-1086;

25 Onodera, Blood 91 (1998), 30-36; Verma, Gene Ther. 5 (1998), 692-699; Nabel, Ann. N.Y. Acad. Sci. 811 (1997), 289-292; Verzeletti, Hum. Gene Ther. 9 (1998), 2243-51; Wang, Nature Medicine 2 (1996), 714-716; WO 94/29469; WO 97/00957, US 5,580,859; US 5,589,466; or Schaper, Current Opinion in Biotechnology 7 (1996), 635-640. The recited nucleic acid molecules and vectors may be designed

30 for direct introduction or for introduction via liposomes, or viral vectors (e.g., adenoviral, retroviral) into the cell. Preferably, said cell is a germ line cell,

embryonic cell, or egg cell or derived therefrom, most preferably said cell is a stem cell. An example for an embryonic stem cell can be, inter alia, a stem cell as described in, Nagy, Proc. Natl. Acad. Sci. USA 90 (1993), 8424-8428.

In accordance with the above, the present invention relates to methods to derive  
5 vectors, particularly plasmids, cosmids, viruses and bacteriophages used conventionally in genetic engineering that comprise a nucleic acid molecule encoding the polypeptide sequence of a bispecific single chain antibody constructs defined herein. Preferably, said vector is an expression vector and/or a gene transfer or targeting vector. Expression vectors derived from viruses such as  
10 retroviruses, vaccinia virus, adeno-associated virus, herpes viruses, or bovine papilloma virus, may be used for delivery of the recited polynucleotides or vector into targeted cell populations. Methods which are well known to those skilled in the art can be used to construct recombinant vectors; see, for example, the techniques described in Sambrook et al. (loc cit.), Ausubel (1989, loc cit.) or other standard  
15 text books. Alternatively, the recited nucleic acid molecules and vectors can be reconstituted into liposomes for delivery to target cells. The vectors containing the nucleic acid molecules of the invention can be transferred into the host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas  
20 calcium phosphate treatment or electroporation may be used for other cellular hosts; see Sambrook, supra.

The recited vector may be the pEF-DHFR, pEF-ADA or pEF-neo.

The vectors pEF-DHFR and pEF-ADA have been described in the art, e.g. in Mack et al. (PNAS (1995) 92, 7021-7025) and Raum et al. (Cancer Immunol Immunother  
25 (2001) 50(3), 141-150).

It is further envisaged that the pharmaceutical composition of the invention comprises a host transformed or transfected with a vector defined herein above.

Said host may be produced by introducing said at least one of the above described  
30 vector or at least one of the above described nucleic acid molecules into the host. The presence of said at least one vector or at least one nucleic acid molecule in

the host may mediate the expression of a gene encoding the above described bespecific single chain antibody constructs.

The described nucleic acid molecule or vector which is introduced in the host may either integrate into the genome of the host or it may be maintained  
5 extrachromosomally.

The host can be any prokaryote or eukaryotic cell.

The term "prokaryote" is meant to include all bacteria which can be transformed or transfected with a DNA or RNA molecules for the expression of a protein of the invention. Prokaryotic hosts may include gram negative as well as gram positive  
10 bacteria such as, for example, *E. coli*, *S. typhimurium*, *Serratia marcescens* and *Bacillus subtilis*. The term "eukaryotic" is meant to include yeast, higher plant, insect and preferably mammalian cells. Depending upon the host employed in a recombinant production procedure, the protein encoded by the polynucleotide of the present invention may be glycosylated or may be non-glycosylated. Especially  
15 preferred is the use of a plasmid or a virus containing the coding sequence of the polypeptide of the invention and genetically fused thereto an N-terminal FLAG-tag and/or C-terminal His-tag. Preferably, the length of said FLAG-tag is about 4 to 8 amino acids, most preferably 8 amino acids. An above described polynucleotide can be used to transform or transfect the host using any of the techniques  
20 commonly known to those of ordinary skill in the art. Furthermore, methods for preparing fused, operably linked genes and expressing them in, e.g., mammalian cells and bacteria are well-known in the art (Sambrook, loc cit.).

Preferably, said the host is a bacteria, an insect, fungal, plant or animal cell.

It is particularly envisaged that the recited host may be a mammalian cell, more  
25 preferably a human cell or human cell line.

Particularly preferred host cells comprise CHO cells, COS cells, myeloma cell lines like SP2/0 or NS/0.

The pharmaceutical composition of the invention may also comprise a  
30 proteinaceous compound capable of providing an activation signal for immune effector cells useful for cell proliferation or cell stimulation.

The proteinaceous compound is not understood as an additional domain of the above defined bispecific single chain antibody construct, but at least one additional component of the pharmaceutical composition of the invention.

In the light of the present invention, said "proteinaceous compounds" providing an activation signal for immune effector cells" may be, e.g. a further activation signal for T cells (e.g. a further costimulatory molecule: molecules of the B7-family, Ox40 L, 4.1 BBL), or a further cytokine: interleukin (e.g. IL-2), or an NKG-2D engaging compound. Preferred formats of proteinaceous compounds comprise additional bispecific antibodies and fragments or derivatives thereof, e.g. bispecific scFv.

Proteinaceous compounds can comprise, but are not limited to scFv fragments specific for the T cell receptor or superantigens. Superantigens directly bind to certain subfamilies of T cell receptor variable regions in an MHC-independent manner thus mediating the primary T cell activation signal. The proteinaceous compound may also provide an activation signal for immune effector cell which is a non-T cell. Examples for immune effector cells which are non-T cells comprise, inter alia, NK cells.

An additional technical feature of the pharmaceutical composition of the invention is that said pharmaceutical composition is thermostable at  $\geq 37^{\circ}\text{C}$ :

20

An alternative embodiment of the invention relates to a process for the production of a pharmaceutical composition of the invention, said process comprising culturing a host defined herein above under conditions allowing the expression of the construct and recovering the produced bispecific single chain antibody construct from the culture.

25

The transformed hosts can be grown in fermentors and cultured according to techniques known in the art to achieve optimal cell growth. The polypeptide of the invention can then be isolated from the growth medium, cellular lysates, or cellular membrane fractions. The isolation and purification of the, e.g., microbially expressed polypeptides of the invention may be by any conventional means such as, for example, preparative chromatographic separations and immunological

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separations such as those involving the use of monoclonal or polyclonal antibodies directed, e.g., against a tag of the polypeptide of the invention or as described in the appended examples.

The conditions for the culturing of a host which allow the expression are known in the art and discussed herein above. The same holds true for procedures for the purification/recovery of said constructs.

A further alternative embodiment of the invention relates to the use of a bispecific single chain antibody construct as defined above, a nucleic acid sequence as defined above, a vector as defined above, a host as defined above and/or produced by a process as defined above for the preparation of a pharmaceutical composition for the prevention, treatment or amelioration of a tumorous disease.

In particular, the pharmaceutical composition of the present invention may be particularly useful in preventing, ameliorating and/or treating cancer.

Preferably said tumorous disease is epithelial cancer or a minimal residual cancer.

It is envisaged by the present invention that the above defined bispecific single chain antibody construct, nucleic acid molecules and vectors are administered either alone or in any combination using standard vectors and/or gene delivery systems, and optionally together with a pharmaceutically acceptable carrier or excipient. Subsequent to administration, said nucleic acid molecules or vectors may be stably integrated into the genome of the subject.

On the other hand, viral vectors may be used which are specific for certain cells or tissues and persist in said cells. Suitable pharmaceutical carriers and excipients are well known in the art. The pharmaceutical compositions prepared according to the invention can be used for the prevention or treatment or delaying the above identified diseases.

Furthermore, it is possible to use a pharmaceutical composition of the invention which comprises described nucleic acid molecules or vectors in gene therapy. Suitable gene delivery systems may include liposomes, receptor-mediated delivery systems, naked DNA, and viral vectors such as herpes viruses, retroviruses,

adenoviruses, and adeno-associated viruses, among others. Delivery of nucleic acids to a specific site in the body for gene therapy may also be accomplished using a biolistic delivery system, such as that described by Williams (Proc. Natl. Acad. Sci. USA 88 (1991), 2726-2729). Further methods for the delivery of nucleic acids comprise particle-mediated gene transfer as, e.g., described in Verma, Gene Ther. 15 (1998), 692-699.

Furthermore the invention relates to a method for the prevention, treatment or amelioration of a tumorous disease comprising the step of administering to a subject in the need thereof an effective amount a bispecific single chain antibody construct as defined above, a nucleic acid sequence as defined above, a vector as defined as defined above, a host as defined above and/or produced in by a process as defined above.

Preferably said subject is a human.

The method for the prevention, treatment or amelioration of the invention may comprise the co-administration of an above defined proteinaceous compound capable of an activation signal for immune effector cells to the subject. The co-administration may be a simultaneous co-administration or a non-simultaneous co-administration.

It is particularly preferred for the use and the method of the invention that said tumorous disease is epithelial cancer, preferably adenocarcinomas, or a minimal residual cancer, preferably early solid tumor, advanced solid tumor or metastatic solid tumor.

Finally, the present invention relates to a kit comprising a bispecific single chain antibody construct as defined above, a nucleic acid sequence as defined above, a vector as defined above and/or a host as defined above. It is also envisaged that the kit of this invention comprises a pharmaceutical composition as described herein above, either alone or in combination with further medicaments to be administered to a patient in need of medical treatment or intervention.

The Figures show:

**Figure 1:**

DNA and amino acid sequence of the anti-CD3-anti-EpCAM constructs **A)** anti-  
 5 CD3 VHVL stL x 3-1 VHVL (SEQ ID NO.:11,12), **B)** anti-CD3 VHVL aL x 4-7 VHVL  
 (SEQ ID NO.:1,2), **C)** anti-CD3 VHVL aL Ser x 4-7 VHVL (SEQ ID NO.:7, 8), **D)**  
 anti-CD3 VHVL stL x 4-7 VHVL (SEQ ID NO.:13,14), **E)** anti-CD3 VHVL stL x 4-7  
 VLVH (SEQ ID NO.:15,16), **F)** anti-CD3 VHVL aL x 5-10 VHVL (SEQ ID NO.:3,4),  
**G)** anti-CD3 VHVL aL Ser x 5-10 VHVL (SEQ ID NO.:9, 10), **H)** anti-CD3 VHVL stL  
 0 x 5-10 VHVL (SEQ ID NO.:17,18), **I)** anti-CD3 VHVL stL x 5-10 VLVH (SEQ ID  
 NO.:19,20), **J)** anti-CD3 VHVL aL x 3-1 VHVL (SEQ ID NO.:45, 46), **K)** anti-CD3  
 VHVL aL Ser x 3-1 VHVL (SEQ ID NO.:47,48), **L)** anti-CD3 VHVL aL x 3-5 VHVL  
 (SEQ ID NO.:49,50), **M)** anti-CD3 VHVL aL Ser x 3-5 VHVL (SEQ ID NO.:51,52),  
**N)** anti-CD3 VHVL stL x 3-5 VHVL (SEQ ID NO.:53,54), **O)** anti-CD3 VHVL aL x 4-  
 5 1 VHVL (SEQ ID NO.:55,56), **P)** anti-CD3 VHVL aL Ser x 4-1 VHVL (SEQ ID  
 NO.:57,58) and **Q)** anti-CD3 VHVL stL x 4-1 VHVL (SEQ ID NO.:59,60).

**Figure 2:**

FACS analysis of the constructs **A)** anti-CD3 VHVL stL x 5-10 VHVL (SEQ ID  
 10 NO.:18), **B)** anti-CD3 VHVL stL x 4-7 VHVL (SEQ ID NO.:14), **C)** anti-CD3 VHVL  
 aL x 5-10 VHVL (SEQ ID NO.:4), **D)** anti-CD3 VHVL aL x 4-7 VHVL (SEQ ID  
 NO.:2), **E)** anti-CD3 VHVL aL Ser x 5-10 VHVL (SEQ ID NO.:10), **F)** anti-CD3  
 VHVL aL Ser x 4-7 VHVL (SEQ ID NO.:8), **G)** anti-CD3 VHVL stL x 3-1 VHVL  
 (SEQ ID NO.:12), **H)** anti-CD3 VHVL stL x 5-10 VLVH (SEQ ID NO.:20) and **I)** anti-  
 15 CD3 VHVL stL x 4-7 VLVH (SEQ ID NO.:16) in CD3 positive Jurkat and EpCAM-  
 positive Kato III cells. A shift to the right shows binding. In Jurkat and KatoIII cells  
 the dotted line indicates the shift of the negative control (only secondary antibody),  
 dashed line shows the binding of an anti-EpCAM-anti-CD3 control antibody and the  
 bold line shows the bispecific construct of interest.

30

**Figure 3:**



DNA and amino acid sequence of the anti-EpCAM-anti-CD3- constructs **A)** 4-7 VLVHx anti-CD3 VHVL (SEQ ID NO.:41,42), **B)** 3-5 VLVHx anti-CD3 VHVL (SEQ ID NO.:29,30), **C)** 3-1 VLVHx anti-CD3 VHVL (SEQ ID NO.:35,36), **D)** 4-1 VLVHx anti-CD3 VHVL (SEQ ID NO.:38,39) and **E)** 5-10 VLVHx anti-CD3 VHVL (SEQ ID NO.:43,44).

**Figure 4:** FACS analysis of the constructs **A)** 4-7 VLVHx anti-CD3 VHVL (SEQ ID NO.:42), **B)** 3-5 VLVHx anti-CD3 VHVL (SEQ ID NO.:30), **C)** 3-1 VLVHx anti-CD3 VHVL (SEQ ID NO.:36), **D)** 4-1 VLVHx anti-CD3 VHVL (SEQ ID NO.:39) and **E)** 5-10 VLVHx anti-CD3 VHVL (SEQ ID NO.: 44) constructs in CD3 positive Jurkat and EpCAM-positive Kato III cells. A shift to the right shows binding.

#### Figure 5:

A representative elution pattern of an EpCAM bispecific antibody containing protein fractions from a Zn-Chelating Fractogel® column at 280 nm. High adsorption at 280 nm from 50-450 ml retention time was due to non-bound protein in the column flow through. The arrow at the peak at 530.09 ml indicates the EpCAM bispecific construct containing protein fraction that was used for further purification.

#### Figure 6:

A representative protein elution pattern from a Sephadex® S200 gel filtration column at 280 nm. The protein peak at 82.66 ml containing bispecific antibodies against CD3 and EpCAM corresponds to a molecular weight of ca. 52 kD. Fractions were collected from 40-140 ml retention time.

#### Figure 7

**A)** Cation exchange chromatogram of 3-1 x anti-CD3 (SEQ ID NO.:36) shows the overall charge isoforms of the protein. Cation exchange chromatography was performed on a MiniS® (Amersham) column. After washing with 20 mM MES buffer pH 5.5, the protein was eluted with a gradient of elution buffer containing 1 M NaCl: 0-30% in 60 column volumes. The bispecific construct was eluted at 23,58 ml. Unspecific protein was eluted with 1 M NaCl starting at 50 ml.

B) Cation exchange chromatogram of 5-10 x anti-CD3 (SEQ ID NO.:44) shows the overall charge isoforms of the protein. Cation exchange chromatography was performed as in Figure 7A. The bispecific construct was eluted at a shoulder at 35,77 ml. Unspecific protein was eluted with 1 M NaCl starting at 50 ml.

**Figure 8:**

A) Representative SDS-PAGE analysis of EpCAM bispecific single chain antibody protein fractions. Lane M: Molecular weight marker Lane 1: cell culture supernatant; lane 2: IMAC flow through; lane 3: IMAC wash; lane 4: IMAC eluate; lane 5: purified antibody against EpCAM and CD3 obtained from gel filtration.

B) Representative Western blot analysis of purified EpCAM bispecific single chain antibody protein fractions Lane 1: cell culture supernatant; lane 2: IMAC flow through; lane 3: IMAC wash; lane 4: IMAC eluate; lane 5: purified antibody against EpCAM and CD3 obtained from gel filtration.

**Figure 9:**

Cytotoxicity assay of C-terminal EpCAM binders anti-CD3x3-1 (SEQ ID NO.:46), anti-CD3 x-5-10 (SEQ ID NO.:4), and anti-CD3 x4-7 (SEQ ID NO.:2). CB15 T cell clone and CHO-EpCAM cells were used in an E:T ratio of 5:1. CHO-EpCAM cells were stained with PKH26 dye and the cells were counted after bispecific single chain antibody incubation with FACS analysis.

**Figure 10:**

Cytotoxicity assay of N-terminal EpCAM binders 3-1xanti-CD3 (SEQ ID NO.:36), and 5-10xanti-CD3 (SEQ ID NO.:44). CB15 T cell clone and CHO-EpCAM cells were used in an E:T ratio of 5:1. CHO-EpCAM cells were stained with PKH26 dye and the cells were counted after bispecific single chain antibody incubation with FACS analysis.

**Figure 11:**

- A) Sequence alignment of the CDR3 of the VH chains of EpCAM 3-1 (SEQ ID NO.:80), EpCAM 4-1 (SEQ ID NO.: 88), EpCAM 5-10 (SEQ ID NO.: 96), EpCAM 3-5 (SEQ ID NO.: 84), EpCAM 4-7 (SEQ ID NO.:92), compared with CDR3 of the  
5 VH chain of EpCAM M79, HD70 and 3B10. The NXD motif is depicted as bold.
- B) Comparison of the cytotoxic activity of 3-1xanti-CD3 (SEQ ID NO.: 36), 5-10xanti-CD3 (SEQ ID NO.:44), anti-CD3x4-7 (SEQ ID NO.:2) and anti-CD3x5-10 (SEQ ID NO.:18) with M79Xanti-CD3 and HD70xanti-CD3 controls. PBMC cells and Kato III cells were used in a E:T ratio of 10:1. KatoIII cells were stained with  
10 propidium iodide and the cells were counted after bispecific single chain antibody incubation with FACS analysis.

The invention will now be described by reference to the following biological  
15 examples which are merely illustrative and are not to be construed as a limitation of scope of the present invention.

**Example 1: Cloning and expression of the EpCAM constructs**

A number of constructs comprising anti-CD3 and anti-EpCAM in various structures  
20 and domain arrangements were generated. Anti-EpCAM VH and VL variable domains of the antibodies 3-1 are shown in SEQ ID NO.:79, 80, 81, 82, 3-5 in SEQ ID NO.:83, 84, 85, 86, 4-1 in SEQ ID NO.:87, 88, 89, 90, 4-7 SEQ ID NO.:91, 92, 93, 94 and 5-10 in SEQ ID NO.:95, 96, 97, 98. The constructs are summarized in Table 1.

25

Table 1. anti-CD3-anti-EpCAM and anti-EpCAM-anti-CD3 constructs

SEQ ID NO.: Construct No.	Construct	Domain arrangement	Distinctive feature
<b>anti-CD3xanti-EpCAM constructs</b>			
SEQ ID NO.: 1,2	anti-CD3x4-7	VH-VLXVH-VL	
SEQ ID NO.: 3, 4	anti-CD3x5-10	VH-VLXVH-VL	
SEQ ID NO.: 45,46	anti-CD3x3-1	VH-VLXVH-VL	
SEQ ID NO.: 49,50	anti-CD3x3-5	VH-VLXVH-VL	
SEQ ID NO.: 55,56	anti-CD3x4-1	VH-VLXVH-VL	
SEQ ID NO.: 7,8	anti-CD3x 4-7Cys-Ser	VH-VLXVH-VL	Cys-Ser mutation
SEQ ID NO.: 9,10	anti-CD3x 5-10Cys-Ser	VH-VLXVH-VL	Cys-Ser mutation
SEQ ID NO.: 47,48	anti-CD3x3-1	VH-VLXVH-VL	Cys-Ser mutation
SEQ ID NO.: 51,52	anti-CD3x3-5	VH-VLXVH-VL	Cys-Ser mutation
SEQ ID NO.: 57,58	anti-CD3x4-1	VH-VLXVH-VL	Cys-Ser mutation
SEQ ID NO.: 11,12	anti-CD3x3-1	VH-VLXVH-VL	(G <sub>4</sub> S) <sub>3</sub> -linker
SEQ ID NO.: 13,14	anti-CD3x4-7	VH-VLXVH-VL	(G <sub>4</sub> S) <sub>3</sub> -linker
SEQ ID NO.: 15,16	anti-CD3x4-7	VH-VLXVL-VH	(G <sub>4</sub> S) <sub>3</sub> -linker
SEQ ID NO.: 17,18 1	anti-CD3x5-10	VH-VLXVH-VL	(G <sub>4</sub> S) <sub>3</sub> -linker
SEQ ID NO.: 19,20	anti-CD3x5-10	VH-VLXVL-VH	(G <sub>4</sub> S) <sub>3</sub> -linker
SEQ ID NO.: 53,54	anti-CD3x3-5	VH-VLXVH-VL	(G <sub>4</sub> S) <sub>3</sub> -linker
SEQ ID NO.: 59, 60	anti-CD3x4-1	VH-VLXVH-VL	(G <sub>4</sub> S) <sub>3</sub> -linker
<b>anti-EpCAM- anti-CD3 constructs</b>			
SEQ ID NO.: 29,30	3-5xanti-CD3	VL-VHxVH-VL	
SEQ ID NO.: 35,36	3-1xanti-CD3	VL-VHxVH-VL	
SEQ ID NO.: 38,39	4-1xanti-CD3	VL-VHxVH-VL	
SEQ ID NO.: 41,42	4-7xanti-CD3	VL-VHxVH-VL	
SEQ ID NO.: 43,44	5-10xanti-CD3	VL-VHxVH-VL	

## 1.1 Cloning of C-terminal EpCAM-binders

### 1.1.1 Preparation of anti-CD3 PCR products

#### a) Anti-CD3 constructs with original 18 amino acid linker (SEQ ID NOs.:1, 2, 3 and 4)

5 The N-terminal original anti-CD3 containing the 18 amino acid linker (SEQ ID NO.:70) was obtained by PCR using the CD19xCD3 construct (Löffler A et al., Blood 2000 95:2098-103) as template and the following primers (CD3 VH *BsrGI*: AGGTGTACACTCCGATATCAAAGTGCAGCAG (SEQ ID NO.:5), CD3 VL *BspEI*: AATCCGGATTTCAGCTCCAGCTTGG (SEQ ID NO.:6)).

#### b) Anti-CD3 constructs with original 18 amino acid linker and Cys to Ser mutation in CDRH3 (SEQ ID Nos. 7,8, 9 and 10)

The N-terminal original anti-CD3 containing the 18 amino acid linker (Seq ID NO.:70) and the Cys to Ser mutation was obtained by PCR using a CD19xanti-CD3 (C→S mutation) construct as template and the primers CD3 VH *BsrGI* and  
 15 CD3 VL *BspEI* (Seq ID Nos. 5 and 6). The CDRH3 sequence with the Cys-Ser mutation is shown in SEQ ID NO.:78.

#### c) Anti-CD3-anti-EpCAM constructs with (G<sub>4</sub>S)<sub>3</sub> linker (Seq ID Nos. 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20)

The N-terminal anti-CD3 containing the 15 amino acid standard (G<sub>4</sub>S)<sub>3</sub> linker (SEQ  
 20 ID NO.:99) was obtained by PCR using the CD19xCD3 (Löffler A et al., Blood 2000 95:2098-103) as template. The anti-CD3 VH region and the anti-CD3 VL region were separately amplified by the following primers (CD3 VH: CD3 VH *BsrGI* AGGTGTACACTCCGATATCAAAGTGCAGCAG (SEQ ID NO.:5), 3'CD3 VH GS15 GGAGCCGCCGCCGCGCCAGAACCACCACCACCTGAGGAGACTGTGA  
 25 GAGTGGTGCCTTG (SEQ ID NO.:21); CD3 VL: 5'CD3 VL GS15 GGCGGCGGCGGCTCCGGTGGTGGTGGTTCTGACATTCAGC TGACCCAGTCTCC (SEQ ID NO.:22), CD3 VL *BspEI* AATCCGGATTTCAGCTCCAGCTTGG (SEQ ID NO.:6)). Overlapping complementary sequences introduced into the PCR products were used to form the  
 30 coding sequence of a 15-amino acid (G<sub>4</sub>S)<sub>3</sub> (single-letter amino acid code) (SEQ ID

NO.:99) linker during the subsequent fusion PCR. This amplification step was performed with the primer pair CD3 VH *BsrGI* (SEQ ID NO.:5) and CD3 VL *BspEI* (SEQ ID NO.:6).

### 1.1.2 Cloning of the anti-CD3xanti EpCAM constructs in $VH_{\text{anti-CD3}}-VL_{\text{anti-CD3}} \times$

5  $VH_{\text{anti-EpCAM}}-VL_{\text{anti-EpCAM}}$  orientation (SEQ ID NO.:1,2, SEQ ID NO.:3,4, SEQ ID NO.:7,8, SEQ ID NO.:9,10, SEQ ID NO.:11,12, SEQ ID NO.:13,14 and SEQ ID NO.:17,18)

The N-terminal original anti-CD3 containing the 18 amino acid linker (SEQ ID NO.:70) or the N-terminal original anti-CD3 containing the 15 amino acid standard (G<sub>4</sub>S)<sub>3</sub> linker (SEQ ID NO.:99) was cleaved with the restriction enzymes *BsrGI* and *BspEI* and subsequently cloned into the bluescript KS vector (Stratagene, La Jolla, CA), containing the amino acid sequence of an eukaryotic secretory signal (leader peptide) as a *EcoRI/BsrGI*-Fragment. After cleavage of this construct with *EcoRI* and *BspEI* the resulting DNA fragment comprising the respective anti-CD3 scFv  
10 with the leader peptide was cloned into a *EcoRI/BspEI* cleaved plasmid containing the c-terminal EpCAM binders 3-1 (SEQ ID NO.:79-82), 4-7 (SEQ ID NO.:91-94), or 5-10 (SEQ ID NO.:95-98) in pEFDHFR. pEFDHFR was described in Mack et al. Proc. Natl. Acad. Sci. USA 92 (1995) 7021-7025).

### 1.1.3. Cloning of the anti-CD3xanti EpCAM constructs in $VH_{\text{anti-CD3}}-VL_{\text{anti-CD3}} \times$

20  $VL_{\text{anti-EpCAM}}-VH_{\text{anti-EpCAM}}$  orientation (SEQ ID Nos.: 15, 16, 19 and 20)

The C-terminal anti-EpCAM antibody 4-7 (SEQ ID NO.:91-94) in VLVH orientation containing the 15 amino acid standard linker (SEQ ID NO.:99) was obtained by PCR. The 4-7 VH region and the 4-7 VL region were separately amplified by the following primers (4-7 -VL: 4-7 VL *BspEI* FOR  
35 CTGAAATCCGGAGGTGGTGGATCCGAGCTCGTGATGACCCAGACTCC (SEQ ID NO.:100), 4-7 VL GS15 REV GGAGCCGCCGCCGCGCCAGAACCACCA CCACCTTTGATCTCAAGCTTGGTCCCC (SEQ ID NO.:101); 4-7 VH: 4-7 VH GS15 FOR  
GGCGGCGGCGGCTCCGGTGGTGGTGGTTCTGAGGTGCAGCTGCTCGAGCA  
40 G (SEQ ID NO.:23), 4-7 VH *SaI* REV TTTTAAGTCGACCTAATGATGATGAT-

GATGATGTGAGGAGACGGTGACCGTGG (SEQ ID NO.:24)). Overlapping complementary sequences introduced into the PCR products were used to form the coding sequence of a 15-amino acid (G<sub>4</sub>S)<sub>3</sub> (single-letter amino acid code) linker (SEQ ID NO.:99) during the subsequent fusion PCR. This amplification step was performed with the primer pair 4-7 VL *Bsp*El FOR and 4-7 VH *Sa*II REV (SEQ ID NO.100, SEQ ID NO.:24).

The C-terminal anti-EpCAM antibody 5-10 (SEQ ID NO.:95-98) in VLVH orientation containing the 15 amino acid standard linker (SEQ ID NO.:99) was obtained by PCR. The 5-10 VH region and the 5-10 VL region were separately amplified by the following primers (5-10 VL: 5-10 VL *Bsp*El FOR CTGAAATCCGGAGGTGGTGGATCCGAGCTCGTGATGACACAGTCTCCAT (SEQ ID NO.:25), 5-10 VL GS15 REV GGAGCCGCCGCCGCGCCAGAACCACCACCACCTTTGATCTCAAGCTTGGTCCCA G (SEQ ID NO.: 26); 5-10 VH: 5-10 VH GS15 FOR GGCGGCGGCGGCTCCGGTGGTGGTGGTTCTGAGGTGCAGCTGCTCGAGC (SEQ ID NO.:27), 5-10 VH *Sa*II REV TTTTAAGTCGACCTAATGATGATGATGATGTGAGGAGACGGTGACCGTG G (SEQ ID NO.:28)). Overlapping complementary sequences introduced into the PCR products were used to form the coding sequence of a 15-amino acid (G<sub>4</sub>S)<sub>3</sub> linker (SEQ ID NO.:99) during the subsequent fusion PCR. This amplification step was performed with the primer pair 5-10 VL *Bsp*El FOR and 5-10 VH *Sa*II REV (SEQ ID NO.:25, SEQ ID NO.:28).

These PCR products (5-10 VLVH and 4-7 VLVH) were cleaved with *Bsp*El and *Sa*II and ligated in the *Bsp*El/*Sa*II cleaved anti-CD3 VHVL stLx5-10 VHVL (SEQ ID NO.:17,18) or anti-CD3 VHVL stL x 4-7 (SEQ ID NO.:13, 14) VHVL in pEFDHFR replacing the 5-10 VHVL DNA fragment.

#### 1.1.4. Expression and binding of the anti-CD3-EpCAM constructs

After confirmation of the sequence coding for the bispecific single chain by sequencing the plasmid was transfected into DHFR deficient CHO cells for eukaryotic expression. Eukaryotic protein expression in DHFR deficient CHO cells

was performed as described in Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566). The transfected cells were then expanded and 1 litre of supernatant produced. Expression and binding of the bispecific single chain molecules were confirmed by FACS analyses. For that purpose the EpCAM positive human gastric cancer cell line Kato III (obtained from American Type Culture Collection (ATCC) Manassas, VA 20108 USA, ATCC number: HTB-103) was used. Binding of the anti-CD3 part was demonstrated on Jurkat cells (ATCC TIB 152).

Cells were cultured according to the recommendations of the supplier and ca. 200000 cells were incubated with 10µg/ml of the construct in 50µl PBS with 2%FCS. The binding of the construct was detected with an anti-His antibody (Penta-His Antibody, BSA free, obtained from Quiagen GmbH, Hilden, FRG) at 2µg/ml in 50µl PBS with 2%FCS. As a second step reagent a R-Phycoerythrin-conjugated affinity purified F(ab')<sub>2</sub> fragment, goat anti-mouse IgG, Fc-gamma fragment specific antibody, diluted 1:100 in 50µl PBS with 2% FCS (obtained from Dianova, Hamburg, FRG) was used. The samples were measured on a FACSScan (BD biosciences, Heidelberg, FRG). All the constructs comprising anti-CD3 and anti-EpCAM showed stronger binding affinity to CD3 and to EpCAM than the prior art anti-EpCAM (M79)xanti-CD3 bispecific antibody (Figure 2).

## 1.2 N-terminal EpCAM binders

### 1.2.1 Cloning of the anti-EpCAMxanti-CD3 constructs

#### Cloning of the construct 3-5xanti-CD3 (SEQ ID NOs.29, 30):

The C-terminal 3-5 in VH-VL orientation was obtained by PCR for the construction of 3-5 xanti-CD3 (SEQ ID NO.:29) molecule. Fragments I and II were amplified by PCR using primer pairs me 81 (SEQ ID NO.:31) /me 90 (SEQ ID NO.:34) and me 83 (SEQ ID NO.:32) /me 84 (SEQ ID NO.:33), respectively. Hot Start PCR was done using the Expand High Fidelity System of Roche Diagnostics. 20 cycles (94°C/30 sec; 60°C/1min;72°C/1min) were used for amplification followed by one cycle of 3 min at 72°C.

PCR fragments I and II were subjected to electrophoresis on a 1.5% agarose gel.

Fragments were mixed (1 ng of each) and used as a template for the next PCR



reaction performed with primer pair me 81 (SEQ ID NO.:31) and me 84 (SEQ ID NO.:33) for amplification of fragment III. PCR was performed as described above. Fragment III was purified on an agarose gel and digested with BssHII and BspEI (Biolabs), purified and subsequently cloned into the corresponding sites of the

5 pEF-dHFR-signal peptide (77/78)- anti-CD3 cloning vector, which facilitates cloning of anti-target variable regions in front of the anti-CD3 region. The vector has a unique BssHII site just after the signal peptide followed by BspEI site, linker (G<sub>4</sub>S) and anti-CD3 region. The cloned region was verified by restriction digests and by DNA-sequencing.

10 Sequences of the Primers used:

Me 81: 5'- GGA TGC GCG CGA GCT CGT GAT GAC CCA GAC TCCA CTC TCC -3' (SEQ ID NO.:31)

Me 83: 5'- GGT TCT GGC GGC GGC GGC TCC GGT GGT GGT GGT TCT GAG GTG CAG CTG CTC GA CAG TCT G -3' (SEQ ID NO.:32)

15 Me 84: 5'- GTG CTC CGG AGG AGA CGG TGA CCG TGG TCC CTT GGC CCC AG -3' (SEQ ID NO.:33)

Me 90: 5'- CCG GAG CCG CCG CCG CCA GAA CCA CCA CCA CCT TTG ATC TCA AGC TTG GTC CC -3' (SEQ ID NO.:34)

Cloning of the construct 3-1xanti-CD3 (SEQ ID NO.:35, 36):

20 The C-terminal 3-1 in VH-VL orientation was obtained by PCR for the construction of 3-1 xanti-CD3 (SEQ ID NO.:35) molecule. Fragments I and II were amplified by PCR using primer pairs me 91a (SEQ ID NO.:37) /me 90 (SEQ ID NO.:34) and me 83 (SEQ ID NO.:32) /me 84 (SEQ ID NO.:33) , respectively. PCR was performed as above.

25 Agarose gel fragments comprising PCR fragments I and II were used as a template for the next PCR reaction performed with primer pair me 91a (SEQ ID NO.:37) and me 84 (SEQ ID NO.:33) for amplification of fragment III. PCR was performed as described above except, annealing was performed at 68°C instead of at 60°C. Fragment III was purified on an agarose gel and digested with BsrGI and BspEI

(Biolabs), purified and subsequently cloned into the corresponding sites of the pEF-dHFR-M79 X anti-CD3 cloning vector. The cloned region was verified by restriction digests and by DNA-sequencing.

Me 91a: 5'- GGA TTG TAC A CTCC GA GCT CGT CAT GAC CCA GTC TCC ATC  
5 TTA TCT TGC TGC -3' (SEQ ID NO.:37)

Cloning of the construct 4-1xanti-CD3 (SEQ ID NO.:38, 39) :

The C-terminal 4-1 in VH-VL orientation was obtained by PCR for the construction of 4-1 xanti-CD3 (SEQ ID NO.:38, 39) molecule. Fragments I and II were amplified by PCR using primer pairs me 92a (SEQ ID NO.:40) /me 90 (SEQ ID NO.:34) and  
10 me 83 (SEQ ID NO.:32) /me 84 (SEQ ID NO.:33), respectively. PCR was performed as above in annealing temperature of 60°C.

Agarose gel fragments comprising PCR fragments I and II were used as a template for the next PCR reaction performed with primer pair me 92a (SEQ ID NO.:40) and me 84 (SEQ ID NO.:33) for amplification of fragment III. PCR was performed as  
15 described above except, annealing was performed at 68°C instead of at 60°C. Fragment III was purified on an agarose gel and digested with BsrGI and BspEI (Biolabs), purified and subsequently cloned into the corresponding sites of the pEF-dHFR-M79 X anti-CD3 cloning vector. The cloned region was verified by restriction digests and by DNA-sequencing.

20 Me 92a: 5'- GGA TTG TAC A CTCC GA GCT CGT GAT GAC ACA GTCTCC ATC CTC C -3' (SEQ ID NO.:40)

Cloning of the construct 4-7xanti-CD3 (SEQ ID NO.:41,42)

The C-terminal 4-7 in VH-VL orientation was obtained by PCR for the construction of 4-7 xanti-CD3 (SEQ ID NO.:41, 42) molecule. Fragments I and II were amplified  
25 by PCR using primer pairs me 81 (SEQ ID NO.:31) /me 90 (SEQ ID NO.:34) and me 83 (SEQ ID NO.:32) /me 84 SEQ ID NO.:33), respectively. PCR was performed as above with an annealing temperature of 60°C.

Agarose gel fragments comprising PCR fragments I and II were used as a template for the next PCR reaction performed with primer pair me 81 (SEQ ID NO.:31) and

me 84 (SEQ ID NO.:33) for amplification of fragment III. PCR was performed as described above. Fragment III was purified on an agarose gel and digested with BssHII and BspEI (Biolabs), purified and subsequently cloned into the corresponding sites of the pEF-dhfr-signal peptide (77/78)- anti-CD3 cloning vector. The cloned region was verified by restriction digests and by DNA-sequencing.

Cloning of the construct 5-10xanti-CD3 (SEQ ID NO.:43, 44) :

The C-terminal 5-10 in VH-VL orientation was obtained by PCR for the construction of 5-10xanti-CD3 (SEQ ID NO.:43, 44) molecule. Fragments I and II were amplified by PCR using primer pairs me 92a (SEQ ID NO.:40) /me 90 (SEQ ID NO.:34) and me 83 (SEQ ID NO.:32) /me 84 (SEQ ID NO.:33), respectively. PCR was performed as above with an annealing temperature of 60°C.

Agarose gel fragments comprising PCR fragments I and II were used as a template for PCR with primer pair me 92a (SEQ ID NO.:40) and me 84 (SEQ ID NO.:33) for amplification of fragment III. PCR was performed as described above except, annealing was performed at 68°C instead of at 60°C. Fragment III was purified on an agarose gel and digested with BsrGI and BspEI (Biolabs), purified and subsequently cloned into the corresponding sites of the pEF-dhfr-M79 X anti-CD3 cloning vector. The cloned region was verified by restriction digests and by DNA-sequencing.

**1.2.2 Expression of anti-EpCAMxanti-CD3 bispecific molecules**

CHO-cells lacking DHFR gene were maintained in alpha MEM medium (Life Technologies, cat.no: 32561) supplemented with 10% fetal Calf Serum (Life Technologies, heat inactivated at 65°C for 30 minutes) and with HT (Hypoxanthin and Thymidine; Life Technologies, cat. no: 41065-012). The cells were transfected with pEF-dHFR-3-1xanti-CD3 (SEQ ID NO.:35, 36), pEF-dHFR-3-5xanti-CD3 (SEQ ID NO.:29, 30), pEF-dHFR-4-1xanti-CD3 (SEQ ID NO.:38, 39), pEF-dHFR-4-7xanti-CD3 (SEQ ID NO.:41, 42) and pEF-dHFR-5-10xanti-CD3 (SEQ ID NO.:43, 44) using Lipofectamine 2000 kit (Invitrogen; cat. no:11668-019) according to the instructions provided by the Manufacturer. After 48 hrs, the cells were subjected to

selection by transferring the transfected cells into the selection medium (alpha MEM medium (cat. no:32561) containing heat inactivated 10% dialysed fetal Calf Serum (Life Technologies). After 2-3 weeks of selection, the cells were grown for 8 to 9 days (in 500 ml of selection medium) for production of bispecific molecules in 2  
5 litre Tissue culture Roller Bottles (Falcon (cat. no: 353068;Becton Dickinson Labware). The tissue culture medium was centrifuged at 4°C for 10 minutes at 300g (1300rpm) to remove the cells and cell debris. The supernatant containing the secreted bispecific molecules was stored at -20°C until further analysis.

### 1.2.3 Binding assays of bispecific anti EpCAMxanti CD3 variants

10 In order to analyze the binding strength of the bispecific anti-EpCAMxanti-CD3 single chain constructs of the invention, the following binding assay was carried out.

250000 Jurkat cells (for CD3 binding) and Kato cells (for EpCAM binding) were independently incubated with crude supernatants (50µl) containing bispecific  
15 construct for 45 min. at 4°C. Thereafter, the cells were washed twice in FACS buffer (phosphate-buffered saline containing 1% fetal calf serum (FCS) and 0.05% sodium azide) and incubated with mouse anti-His antibody (Dianova,DIA910) for 60 min. at 4°C. Washing steps were performed as above.

The cells were finally incubated either with goat anti-mouse-FITC-conjugated  
20 antibody (BD 550003) or with anti-mouse-PE conjugated antibody (IgG) (Sigma, P8547). After washing steps, 10,000 events were analysed using FACS Calibur (B&D). All the EpCAM constructs showed strong binding (Figure 4).

### Example 2. Purification of the EpCAM constructs

25 In order to purify the bispecific single chain constructs comprising anti-EpCAM and anti-CD3 the CHO-EpCAM cells were grown in roller bottles with HiClone® CHO modified DMEM medium (HiQ) for 7 days before harvest. The cells were removed by centrifugation and the supernatant containing the expressed protein was stored at -20°C.

Äkta FPLC System® (Pharmacia) and Unicorn Software® were used for chromatography. All chemicals were of research grade and purchased from Sigma (Deisenhofen) or Merck (Darmstadt).

IMAC was performed, using a Fractogel® column (Pharmacia) that was loaded  
5 with  $\text{ZnCl}_2$  according to the manufacturers protocol. The column was equilibrated  
with buffer A2 (20 mM NaPP pH 7.5, 0.4 M NaCl) and the cell culture supernatant  
(500ml) was applied to the column (10 ml) with a flow rate of 3 ml/min. The column  
was washed with buffer A2 to remove unbound sample. Bound protein was eluted  
using a 2-step gradient of buffer B2 (20 mM NaPP pH 7.5, 0.4 M NaCl, 0.5 M  
10 Imidazol). In Step 1 20% buffer B2 in 10 column volumes was used and in Step2  
100% buffer B2 in 10 column volumes was used. Eluted protein fractions from the  
100% step were pooled for further purification.

Gel filtration chromatography was performed on a Sephadex S200 HiPrep®  
column (Pharmacia) equilibrated with PBS (Gibco). Eluted protein samples (flow  
15 rate 1ml/min) were subjected to SDS-Page and Western Blot for detection.

The column was previously calibrated for molecular weight determination  
(molecular weight marker kit, Sigma MW GF-200).

Protein concentrations were determined using protein assay dye (MicroBCA,  
Pierce) and IgG (Biorad) as standard protein. The yields of the protein are shown  
20 in Table 2. All constructs were producible.

**Table 2. Yields of the single-chain bispecific constructs comprising anti-EpCAM and anti-CD3**

Construct	Yield [ $\mu$ g purified protein per liter culture]
4-1 x anti-CD3 (SEQ ID NO.:39)	172.5
3-5 x anti-CD3 (SEQ ID NO.:30)	265
4-7 x anti-CD3 (SEQ ID NO.:42)	37
anti-CD3 x 4-7. (SEQ ID NO.:2)	112.5
anti-CD3 Cys-Ser x 4-7 (SEQ ID NO.:8)	140
3-1 x anti-CD3 (SEQ ID NO.:36)	265
5-10 x anti-CD3 (SEQ ID NO.:44)	400
anti-CD3 x 5-10 (SEQ ID NO.:4)	195

- A further high resolution cation exchange chromatography was performed on a MiniS® column (Amersham), equilibrated with 20mM MES buffer pH 5.5. The sample was diluted 1:3 with the same buffer before loading to the column. Bound protein was eluted with a gradient of equilibration buffer containing 1M NaCl: 0-30% in 60 column volumes. Remaining protein was eluted in 3 column volumes of 1M NaCl (**Figure 7**).
- The EpCAM bispecific single chain construct proteins were isolated in a two-step purification process including immobilized metal affinity chromatography (IMAC) (**Figure 5**) and gel filtration (**Figure 6**). The main product had a molecular weight of 52 kDa under native conditions as determined by gelfiltration in PBS.
- Purified bispecific protein was analyzed in SDS PAGE under reducing conditions performed with precast 4-12% Bis Tris gels (Invitrogen). Sample preparation and application were according to the manufacturers protocol. The molecular weight was determined with MultiMark® protein standard (Invitrogen). The gel was stained with colloidal Coomassie (Invitrogen protocol). The purity of the isolated protein was shown to be >95% (**Figure 8A**). Western Blot was performed with an Optitrans BA-S83® membrane and the Invitrogen Blot Module according to the manufacturers protocol. The antibodies used were Penta His (Qiagen) and Goat-anti-Mouse-Ig labeled with alkaline phosphatase (AP) (Sigma), the chromogenic

substrate solution was BCIP/NBT liquid (Sigma). The EpCAM bispecific protein could be specifically detected by Western Blot (**Figure 8B**). The main signal corresponds to the main band in the SDS PAGE at 52 kD corresponding to the purified bispecific molecule.

5

**Example 3. Cytotoxicity assays of the constructs comprising anti-CD3 and anti-EpCAM**

In order to test the bioactivity of the constructs comprising anti-EpCAM and anti-CD3 a FACS based cytotoxicity test was performed.

- 10 For the cytotoxicity test, CHO cells from the American Type Cell Culture Collection (ATCC, Manassas, USA) were transfected with epithelial cell adhesion molecule (EpCAM). A cell clone derived from this transfection, referred to as CHO-EpCAM cells, was used for the experiments. CHO-EpCAM ( $1.5 \times 10^7$ ) cells were washed free of serum two times with PBS and incubated with PKH26 dye (Sigma-Aldrich
- 15 Co.) according to the manufacturers instructions. After staining cells were washed two times with RPMI/10% FCS.

- Cells were counted and mixed with CB15 effector cells. The CD4-positive T cell clone CB15 was provided by Dr. Fickenscher, University of Erlangen/Nuernberg, Germany. Cells were cultured as recommended by the suppliers. The resulting cell
- 20 suspension contained 400.000 target and  $2 \times 10^6$  effector cells per ml. 50  $\mu$ l of the mixture was used per well in a 96 well round bottom plate.

- Antibodies were diluted in RPMI/10% FCS to the required concentration and 50  $\mu$ l of this solution was added to the cell suspension. A standard reaction was incubated for 16 h at 37°C / 5% CO<sub>2</sub>. Propidium iodide was added to a final
- 25 concentration of 1  $\mu$ g/ml. After 10 min of incubation at room temperature cells were analysed by FACS. PKH26 fluorescence was used for positive identification of target cells. Cytotoxicity was measured as ratio of PI positive over all target cells. Sigmoidal dose response curves typically had  $R^2$  values >0.97 as determined by Prism Software (GraphPad Software Inc., San Diego, USA) (**Figure 9 and 10**).

EC<sub>50</sub> values calculated by the analysis program were used for comparison of bioactivity. All the constructs of the invention show at least 50 times better cytotoxicity (maximum EC<sub>50</sub>-value 169 pg/ml) than the prior art construct M79xanti-CD3 (8628 pg/ml).

5

**Example 4. Determination of the binding affinity by BIAcore™ 2000 of the constructs comprising anti-EpCAM and anti-CD3 to EpCAM**

In order to show the superior binding affinity of the constructs of the invention, the KD values of the constructs and of the prior art anti-EpCAM construct (M79)xanti-CD3 were determined.

Kinetic binding experiments were performed using surface plasmon resonance on the BIAcore™ 2000, Biacore AB (Uppsala, Sweden) with a flow rate of 5 µL/min and HBS-EP (0.01 M HEPES, pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% surfactant P20) as running buffer at 25 °C. The extracellular domain of the EpCAM antigen (residues 17-265) was immobilized onto flow cells 2-4 on a CM5 sensor chip. The chip surface was activated injecting 80 µL of 0.1 M sodium-hydroxysuccinimid, 0.4 M N-ethyl-N'(3—dimethylaminepropyl)-carbodiimid (NHS/EDC). The antigen was coupled by manual injection of 60 µg/mL EpCAM in 0.01 M sodium-acetate, pH 4.7. Different densities of antigen were immobilized on flow cells 2-4 adjusting the amount of manual injection times. Flow cell 1 was left empty while flow cell 2 was coated with the highest density of EpCAM (4100 RU). Flow cell 3 was coated with ¼ of the amount of antigen immobilized on flow cell 2 (974 RU) and flow cell 4 was coated with lowest density of Ep-CAM antigen (265 RU). The activated surface of the sensor chip was blocked injecting 85 µL of 1 M ethanolamine and the chip was left to equilibrate over night at a constant flow of 5 µL/min of HBS-EP.

Binding kinetics of the bispecific constructs were measured injecting 10 µL of protein solution at concentrations ranging from 4 µM-0.07 µM and monitoring the dissociation for 100 sec. Protein was buffered in HBS-EP. The data were fitted using BIAevaluation™ software determining the rate constant for dissociation and



association kinetics with a 1:1 Langmuir binding equation (1, 2). Where A is the concentration of injected analyte and B[0] is Rmax.

$$dB/dt = -(ka * [A] * [B] - kd * [AB]) \quad (1)$$

$$dAB/dt = -(ka * [A] * [B] - kd * [AB]) \quad (2)$$

- 5 Kinetic binding curves were determined in four concentrations of each bispecific construct analysed. The independent fitting of the raw data resulted in dissociation and association rate constants that were used to calculate the equilibrium dissociation constant (KD). The calculated KD values were unbiased for concentration indicating reliable data analysis. The average of the independently  
10 determined dissociation constants as well as the standard deviation are summarized in table 3.

The analysed bispecific constructs bind to the Ep-CAM antigen immobilized on the chip surface within a well defined affinity range. The standard deviation for the calculated average dissociation constant is as expected.

15

**Table 3: Dissociation constants for the bispecific constructs binding to EpCAM.**

	KD (M)
M79 x anti-CD3 (control)	4,0x10 <sup>-6</sup>
4-1 x anti-CD3 (SEQ ID NO.:39)	2,5x10 <sup>-7</sup>
3-5 x anti-CD3 (SEQ ID NO.:30)	2,3 x10 <sup>-7</sup>

- The prior art anti-EpCAM x anti-CD3 construct M79xCD3 had a KD of 4,0x10<sup>-6</sup> M  
20 while surprisingly the constructs of the invention have a KD in the range of 2,3 x10<sup>-7</sup> - 2,5 x10<sup>-7</sup> M. Thus, the constructs of the invention have more than 15 times stronger binding affinity than the prior art construct.

**Example 5. Comparison of the cytotoxic activity of the constructs of the invention with prior art constructs**

In order to compare the bioactivity of constructs having the NXD motif with  
5 conventional M79xCD3 and HD70xCD3 constructs the following cytotoxic assay was carried out.

Katolli cells (ATCC HTB-103) were used as target cells and grown in RPMI supplemented with 10% fetal calf serum at 37°C in a 5% CO<sub>2</sub> humidified incubator. Subconfluent cultures were treated with 0.25% trypsin, counted in a Neubauer  
10 chamber slide and checked for vitality by trypan-blue exclusion. Only cultures with >95% vitality were used for cytotoxicity assays. Target cells were stained with PKH26 fluorescent membrane dye according to the manufacturers manual (Sigma-Aldrich GmbH, Germany, PKH26-GL). Cell number was adjusted to  $8 \times 10^5$  cells/ml in RPMI complete medium.

15 Human peripheral blood mononuclear cells (PBMCs) were used as effector cells and isolated from healthy donors using ficoll density gradient centrifugation with subsequent 100 x g centrifugation to remove thrombocytes. The pellet was resuspended in 10 vol. erythrocyte lysing buffer and incubated at room  
20 temperature for 10 min. Lysing reaction was stopped by addition PBS. PBMCs were resuspended in RPMI 1640 complete medium and cell number adjusted to  $8 \times 10^6$  cells/ml.

Equal volumes of target and effector cell suspension were mixed and 50 µl of this  
25 suspension transferred to each well of a 96 well round bottom plate, 50 µl of EpCAM bispecific antibody serial dilution or RPMI complete medium as a negative control was added. Plates were incubated for 16 to 20 hrs at 37°C, 5% CO<sub>2</sub> in a humidified incubator. 50 µl propidium iodide was added to a final concentration of 1 µg/ml and incubated 15 min at room temperature. Samples were analysed by flow  
30 cytometry (FACSCalibur, Becton Dickinson).  $2 \times 10^4$  events were counted.

Target cells were identified by their PKH26 fluorescent label and cytotoxicity within this population was subsequently determined. Viable cells were separated from dead cells by propidium iodide staining and the percentage of dead target cells was used as a measure for cytotoxicity. Mean values were plotted against the concentration of the bispecific antibody on a logarithmic scale, resulting in a dose response curve (Figure 11B). The corresponding EC<sub>50</sub> values were obtained after nonlinear fitting of data with the GraphPad Prism software.

The cytotoxic activity of constructs having the NXD motif (SEQ ID NO.:36, 44, 2 and 18) was compared with conventional constructs M79xanti-CD3 and HD70-xanti-CD3 (Fig. 11B). A sequence alignment of the CDR3 regions of the VH chains of 3-1, 5-10, 4-7, 3-5 and 4-1 with M79, HD70 and 3B10 is shown in Figure 11A. Only 3-1, 5-10, 4-7, 3-5 and 4-1 have the NXD motif and furthermore, the lengths of the CDR3 regions differ. As can be seen from Figure 11A, 3-1, 4-1 and 5-10 have a CDR-H3 region of 10 amino acids, 3-5 and 4-7 have 14 amino acids whereas the prior art M79 has 8 amino acids, 3B10 has 6 amino acids and HD70 has 18 amino acids.

SEQ ID NO.: 36, 44, 2 and 18 showed a clearly better bioactivity compared to the conventional M79 and HD70 constructs (2250 pg/ml and less compared to 71460 and 11327 pg/ml of the prior art constructs, respectively) demonstrating the advantageous effects of the constructs of the invention.

## Claims

1. A pharmaceutical composition comprising a bispecific single chain antibody construct, whereby said construct comprises or consists of at least two domains, whereby one of said domains binds to human EpCAM antigen and a second domain binds to human CD3 antigen, wherein said binding domain specific for EpCAM comprises at least one CDR-H3 region comprising the amino acid sequence NXD preferably in position 102 to 104 of SEQ ID NOs: 80, 88 and 96, or preferably in position 106 to 108 of SEQ ID NOs: 84 and 92, wherein X is an aromatic amino acid.
2. The pharmaceutical composition of claim 1, wherein X is W or Y.
3. The pharmaceutical composition of claim 1 or 2, wherein the CDR-H3 comprises at least 9 amino acid residues.
4. The pharmaceutical composition of any of claims 1 to 3, wherein said binding domain specific for EpCAM has a  $K_D$  value of more than  $5 \times 10^{-9}$  M.
5. The pharmaceutical composition of any of claims 1 to 4, wherein said binding domain specific for EpCAM has a  $K_D$  value in a range between  $1 \times 10^{-7}$  and  $5 \times 10^{-9}$  M and said binding domain specific for CD3 has a  $K_D$  value in a range between  $1 \times 10^{-6}$  and  $5 \times 10^{-9}$  M.
6. A pharmaceutical composition comprising a bispecific single chain antibody construct, whereby said construct comprises or consists of at least two domains, whereby one of said at least two domains specifically binds to human EpCAM antigen and a second domain binds to human CD3 antigen, wherein said binding domain specific for EpCAM comprises at least one CDR-H3 region of at least 9 amino acid residues and wherein said binding

domain specific for EpCAM has a  $K_D$  value of more than  $5 \times 10^{-9}$  M.

7. The pharmaceutical composition of any of claims 1 to 6, wherein said binding domain specific for CD3 has a  $K_D$  value of more than  $10^{-7}$  M.

5

8. The pharmaceutical composition of any of claims 1 to 7, wherein the CDR-H3 region comprises at least 14 amino acids.

10

9. The pharmaceutical composition of any of claims 1 to 8, wherein the  $V_H$  chain domains specific for human EpCAM antigen is selected from the group consisting of:

15

(a) an amino acid sequence as shown in any of SEQ ID NO: 80, SEQ ID NO: 84, SEQ ID NO: 88, SEQ ID NO: 92 and SEQ ID NO: 96;

(b) an amino acid sequence encoded by a nucleic acid sequence as shown in SEQ ID NO: 79, SEQ ID NO: 83, SEQ ID NO: 87, SEQ ID NO: 91 and SEQ ID NO: 95;

20

(c) an amino acid sequence encoded by a nucleic acid sequence hybridizing with the complementary strand of a nucleic acid sequence as defined in (b) under stringent hybridization conditions;

(d) an amino acid sequence encoded by a nucleic acid sequence which is degenerate as a result of the genetic code to a nucleotide sequence of any one of (b) and (c).

25

10. The pharmaceutical composition of any of claims 1 to 9, wherein the  $V_L$  chain domains specific for human EpCAM antigen is selected from the group consisting of:

30

(a) an amino acid sequence as shown in any of SEQ ID NO: 82, SEQ ID NO: 86, SEQ ID NO: 90, SEQ ID NO: 94 and SEQ ID NO: 98;

(b) an amino acid sequence encoded by a nucleic acid sequence as shown in SEQ ID NO: 81, SEQ ID NO: 85, SEQ ID NO: 89, SEQ ID NO: 93 and SEQ ID NO: 97;

(c) an amino acid sequence encoded by a nucleic acid sequence

hybridizing with the complementary strand of a nucleic acid sequence as defined in (b) under stringent hybridization conditions;

- (d) an amino acid sequence encoded by a nucleic acid sequence which is degenerate as a result of the genetic code to a nucleotide sequence of any one of (b) and (c).

- 5
11. The pharmaceutical composition of any of claims 1 to 10, wherein the binding domains specific for the CD3 antigen is derived from an antibody selected from the group consisting of: X35-3, VIT3, BMA030 (BW264/56),
- 10 CLB-T3/3, CRIS7, YTH12.5, F111-409, CLB-T3.4.2, TR-66, WT32, SPv-T3b, 11D8, XIII-141, XIII-46, XIII-87, 12F6, T3/RW2-8C8, T3/RW2-4B6, OKT3D, M-T301, SMC2, WT31 and F101.01.
12. The pharmaceutical composition of any of claims 1 to 11, wherein said
- 15 bispecific single chain antibody construct comprises an amino acid sequence selected from the group of
- (a) an amino acid sequence as shown in any of SEQ ID NOs: 2, 4, 8, 10, 12, 14, 16, 18, 20, 30, 36, 39, 42, 44, 46, 48, 50, 52, 54, 56, 58 and 60;
- 20 (b) an amino acid sequence encoded by a nucleic acid sequence as shown in any of SEQ ID NOs: 1, 3, 7, 9, 11, 13, 15, 17, 19, 29, 35, 38, 41, 43, 45, 47, 49, 51, 53, 55, 57 and 59;
- (c) an amino acid sequence encoded by a nucleic acid sequence hybridizing with the complementary strand of a nucleic acid sequence
- 25 as defined in (b) under stringent hybridization conditions;
- (d) an amino acid sequence encoded by a nucleic acid sequence which is degenerate as a result of the genetic code to a nucleotide sequence of any one of (b) and (c).
- 30 13. The pharmaceutical composition comprising a nucleic acid sequence encoding a bispecific single chain antibody construct as defined in any of claims 1 to 12.

14. The pharmaceutical composition comprising a vector which comprises a nucleic acid sequence as defined in claim 13.
- 5 15. The pharmaceutical composition of claim 14, wherein said vector further comprises a regulatory sequence which is operably linked to said nucleic acid sequence defined in claim 13.
16. The pharmaceutical composition of claim 14 or 15, wherein said vector is an  
10 expression vector.
17. A pharmaceutical composition comprising a host transformed or transfected with a vector defined in any of claims 14 to 16.
- 15 18. A pharmaceutical composition according to any of claims 1 to 17, further comprising a proteinaceous compound capable of providing an activation signal for immune effector cells.
19. The pharmaceutical composition of any of claims 1 to 18, wherein the  
20 pharmaceutical composition is thermostable at  $\geq 37^{\circ}\text{C}$ .
20. A process for the production of a pharmaceutical composition according to any of claims 1 to 19, said process comprising culturing a host defined in claim 17 under conditions allowing the expression of the bispecific single  
25 chain antibody construct as defined in any of claims 1 to 12 and recovering the produced bispecific single chain antibody construct from the culture.
21. Use of a bispecific single chain antibody construct as defined in any of claims 1 to 12, a nucleic acid sequence as defined in claim 13, a vector as  
30 defined in any of claims 14 to 16, a host as defined in claim 17 and/or produced in by a process according to claim 20 for the preparation of a pharmaceutical composition for the prevention, treatment or amelioration of

a tumorous disease.

22. A method for the prevention, treatment or amelioration of a tumorous disease, comprising the step of administering to a subject in need of such a prevention, treatment or amelioration a pharmaceutical composition of any of claim 1 to 19.

23. The method of claim 22, wherein said subject is a human.

24. The use of claim 21 or the method of claim 22 or 23, wherein said tumorous disease is epithelial cancer or a minimal residual cancer.

25. A kit comprising a bispecific single chain antibody construct as defined in any of claims 1 to 12, a nucleic acid sequence as defined in claim 13, a vector as defined in any of claims 14 to 16, a host as defined in claim 17 and/or produced in by a process according to claim 20.



Figure 1

## A) anti-CD3 VHVL stL x 3-1 VHVL (SEQ ID NO: 11)

GATATCAAACCTGCAGCAGTCAGGGGCTGAACCTGGCAAGACCTGGGGCCTCAGTGAAGATGTCCTGCAAGACTTC  
TGGCTACACCTTTACTAGGTACACGATGCACCTGGGTAAACACAGAGGCCCTGGACAGGGTCTGGAATGGATTGGAT  
ACATTAAATCCTAGCCGTGTTATACTAATTAACAATCAGAAAGTTCAAGGACAAGGCCACATTGACTACAGACAAA  
TCCTCCAGCACAGCCFACATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATA  
TTATGATGATCATTAATGCTTGAATACTGGGGCCAAAGGCCACTCTCACAGTCTCCTCAGGTGGTGGTGGT  
CTGGCGGGCGGCTCCGGTGGTGGTCTTGACATTCAGCTGACCCAGTCTCCAGCAATCATGTCTGCATCT  
CCAGGGAGAAAGGTCACCATGACCTGCAGAGCCAGTTCAAGTGTAAAGTTACATGAACCTGGTACCAGCAGAAAGTC  
AGGCACCTCCCCAAAAGATGGATTTATGACACATCCAAAAGTGGCTTCTGGAGTCCCCTTATCGCTTCAGTGGCA  
GTGGGTCTGGGACCTCATACTCTCTCACAAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA  
CAGTGGAGTAGTAACCCGCTCACGTTCCGGTGGTGGACCAAGCTGGAGCTGAAATCCGGAGGTGGTGGATCCGA  
GGTGCAAGCTGCTCGAGCAGTCTGGAGCTGAGCTGGTGAAACCTGGGGCCTCAGTGAAGATATCCTGCAAGGCTT  
CTGGATACGCCCTTCACTAACTACTGGCTAGGTGGGTAAAGCAGAGGCCCTGGACATGGACTTGAGTGGATTGGA  
GATCTTTTCCCTGGAAGTGGTAATACTCACTACAAATGAGAGGTTCAAGGGCCAAAGCCACACTGACTGCAGACAA  
ATCCTCGAGCACAGCCTTTATGCAGCTCAGTAGCCTGACATCTGAGGACTCTGCTGTCTATTCTGTGCAAGAT  
TGAGGAACTGGGACGAGGCTATGGACTACTGGGGCCAAAGGACCACGGTCACCGTCTCCTCAGGTGGTGGT  
TCTGGCGGGCGGCTCCGGTGGTGGTGGTCTGAGCTCGTCATGACCCAGTCTCCATCTTATCTTCTGCTGCATC  
TCCCTGGAGAAACCATTAATAATTGCAGGGCAAGTAAGAGCATTAGCAATAATTTAGCCTGGTATCAAGAGA  
AACCTGGGAAAACATAAAGCTTCTTATCTACTCTGGATCCACTTTGCAATCTGGAATTCATCAAGGTTTCAGT  
GGCAGTGGATCTGGTACAGATTTCACTCTCACCATCAGTAGCCTGGAGCCTGAAG

## Figure 1 A) continued

ATTTTGCAATGTATTACTGTCAACAGCATAATGAATATCCGTACACGTTCCGAGGGGGACCAAGCTTGAGATC  
AAACATCATCACCATCATCATTAG

## (SEQ ID NO: 12)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDK  
SSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSGGGGGGGGGGDIQLTQSPAIMAS  
PGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTISKVASGVPRFSGSGGTSYSLTISSMEAEADAATYYCQ  
QWSSNPLTTFGAGTKLELKSGGGGSEVQLLEQSGAELVKPGASVKISCKASGYAFTNYWLGWVKQRPFGHGLEWIG  
DLFPGSGNTHYNERFRGKATLTADKSSSTAFAFMQLSSLTSEDSAVYFCARLRNWDAMDYWGQGTTVTVSSGGGG  
SGGGSGGGGSELVMTQSPSYLAASPGETITINCRASKSISKYLAWYQEKPGKTNKLLIYSGSTLQSGIPSRFS  
GSGSGTDFTLTISSELEPEDFAMYYCQQQHNEYPYTFGGGKLEIKHHHHH

Figure 1

## B) anti-CD3 VHVL aL x 4-7 VHVL (SEQ ID NO:1)

GATATCAAAGTGCAGCAGTCAGGGGCTGAACTGGCAAGACCTGGGGCCTCAGTGAAGATGTCCCTGCAAGACTTC  
TGGCTACACCTTTACTAGGTACACGATGCACCTGGGTAAACAGAGGCCCTGGACAGGGTCTGGAATGGATTGGAT  
ACATTAAATCCTAGCCGTGGTTATACTAATTACAATCAGAAGTTCAAGGACAAGGCCACATTGACTACAGACAAA  
TCCTCCAGCACAGCCTACATGCAACTGAGCAGCCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATA  
TTATGATGATCATTAATGCTTGAATGCTTGAATGCTTGAATGCTTGAATGCTTGAATGCTTGAATGCTTGAAT  
GTGGAGGTTCTGGTGGAGTGGAGGTTCAAGTGGAGTGGAGTGGAGTGGAGTGGAGTGGAGTGGAGTGGAGTGGAG  
TCTGCATCTCCAGGGGAGAAAGTCAACATGACCTGCAGAGCCAGTTCAAGTGTAAAGTTACATGAACCTGGTACCA  
GCAGAAGTCAGGCACCTCCCCCAAAGATGGATTATGACACATCCAAAGTGGCTTCTGGAGTCCCTTATCGCT  
TCAGTGGCAGTGGGCTGGGACCTCATACTCTCACAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTAT  
TACTGCCAACAGTGGAGTAGTAACCCGCTCACGTTCCGTTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGG  
TGGATCCGAGGTGCAGTGG  
GCAAGGCTTCTGGCTACACCTTCACAAACTATGGTTTAAGCTGGTGAAGCAGAGGCCCTGGACAGGTCCTTGAG  
TGGATTGGAGAGGTTTATCCTAGAATTGGTAATGCTTACTACAATGAGAAGTCAAGGGCAAGGCCACACTGAC  
TGCAGACAAATCCTCCAGCACAGGTCATGGAGTCCGAGCCTGACCTCTGAGGACTCTGCGGTCTATTCT  
GTGCAAGACGGGATCCTACGATACTAACTACGACTGGTACTTCGATGTCTGGGGCCAAAGGGACCCAGGTCAAC  
GTCTCCTCAGGTGG  
TCCACTCTCCCTGCT  
GTAATGGAAACACCTATTACATTGGTACCTGCAGAAAGCCAGGCCAGTCTCCAAAG

## Figure 1 B) continued

CTCCTGATCTACAAGTTTCCAAACCGATTTTCTGGGGTCCCAGACAGGTTCA GTGGCAGTGGATCAGGGACAGA  
TTTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGAGTTTATTTCTGCTCTCAAAGTACACATGTTT  
CGTACACGTTCCGAGGGGGACCAAGCTTGAGATCAAACATCATCACCATCATCATAG

## (SEQ ID NO: 2)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGQLEWIGYINPSRGYTNYNQKFKDKATLTTDK  
SSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSVEGGSGSGGVDIQLTQSPAIM  
SASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPRFSGSGSGTSYSLTISSEAEADAATY  
YCQQWSSNPLTFGAGTKLELKSGGGGSEVQLLEQSGAELARPGASVKLSCKASGYFTFTNYGLSWVKQRPQVLE  
WIGEVYPRIGNAYYNEKFKGKATLTADKSSSTASMELRSLTSEDSAVYFCARRGSYDTNYDWFVWVGQGTVT  
VSSGGGSGGGGSELVMTQTPSLPVSIGDQASISCRSSQSLVHSNGNTYLHWYLOKPGQSPKLLIYKV  
SNRFSGVDPDRFSGSGGTDFTLKISRVEAEDLGVYFCSSQSTHVPYTFGGGTKEIKHHHHH

Figure 1

## C) anti-CD3 VHVL aL Ser x 4-7 VHVL (SEQ ID NO: 7)

GATATCAAACGTCAGCAGTCAGGGGCTGAACCTGGGACCTGGGGCCTCAGTGAAGATGTCTCTGCAAGACTTC  
TGGCTACACCTTTACTAGGTACACGATGCACCTGGGTAAACACAGAGGCCCTGGACAGGGTCTGGAATGGATTGGAT  
ACATTAAATCCTAGCCGTGTTATACTAATAATCAATCAGAAAGTTCAAGGACAAGGCCACATTGACTACAGACAAA  
TCCTCCAGCACAGCCTACATGCAACTGAGCAGCCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATA  
TTATGATGATCATTAATCTCCCTTGACTACTGGGGCCAAAGGCACACTCTCACAGTCTCCTCAGTCGAAGGTGGAA  
GTGGAGGTTCTGGTGGAAGTGGAGGTTCAAGGTGGAGTCGACGACATTCAGCTGACCCAGTCTCCAGCAATCATG  
TCTGCATCTCCAGGGGAGAAGTCAACATGACCTGCAGAGCCAGTTCAAAGTGAAGTTACATGAACCTGGTACCA  
GCAGAAGTCAGGCACCTCCCCCAAAGATGGATTATGACACATCCAAAGTGGCTTCTGGAGTCCCTTATCGCT  
TCAGTGGCAGTGGGTCTGGGACCTCATACTCTCTCACAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTAT  
TACTGCCAACAGTGGAGTAGTAACCCGCTCACGTTCCGTTGCTGGACCAAGCTGGAGCTGAAATCCGGAGGTGG  
TGGATCCGAGGTGCAGCTGCTCGAGCAGTCTGGAGCTGAGCTGGCGAGGCCCTGGGGCTTCAGTGAAGCTGTCCCT  
GCAAGGCTTCTGGCTACACCTTCACAAACTATGGTTTAAGCTGGGTGAAGCAGAGGCCCTGGACAGGTCCTTGAG  
TGGATTGGAGAGGTTATCCTAGAAATGGTAATGCTTACTACAAATGAGAAGTTCAAGGCAAGGCCACACTGAC  
TGCAGACAAATCCTCCAGCACAGCGTCCATGGAGCTCCGCAGCCTGACCTCTGAGGACTCTGCCGTCTATTCT  
GTGCAAGACGGGATCCTACGATACTAACTACGACTGTTACTCGATGCTGGGGCCAAAGGACCAAGGTCAAC  
GTCTCCTCAGGTGGTGGTTCTGGCGGGCGGCTCCGGTGGTGGTTCTGAGCTCGTGATGACCCAGAC  
TCCACTCTCCCTGCTGTCAGTCTTGGAGATCAAGCCCTCCATCTCTTGCAGATCTAGTCAGAGCCTTGTACACA  
GTAATGGAACACCTATTTACATTTGGTACCTGCAGAAGCCAGGCCAGTCTCCAAAGCTCCTGATCTACAAAAGTT  
TCCAACCGATTCTCTGGGGTCCAGACAGGTTTCAGTGGCAGTGGATCAGGGACAG

Figure 1 C) continued

ATTTCACACTCAAGATCAGCAGAGTGAGGCTGAGGATCTGGAGTTTATTCTGCTCTCAAAGTACACATGTT  
CCGTACACGTTCCGAGGGGGACCAAGCTTGAGATCAAAACATCATCACCATCATCATTAG

(SEQ ID NO: 8)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKORPGQGLEWIGYINPSRGYTNYNQKFKDKATLTDDK  
SSSTAYMQLSSLTSEDSAVYYCARYYDDHYSLDYWGQGTTLTVSSVEGGSGGSGGVDDIQLTQSPAIM  
SASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPIYRFSGSGSGTSYSLTISSMEEAEDAATY  
YCQQWSSNPLTFGAGTKLELKSGGGSEVQLLEQSGAELARPGASVKLSCKASGYFTNRYGLSWVKORPGQVLE  
WIGEVYPRIGNAYYNEKFKGKATLTADKSSSTASMELRSLTSEDSAVYFCARRGSDYDNYDWFYFDVWGQGTIVT  
VSSGGGGSGGGSGGSELVMTQTPLSLPVSLGDAQASISCRSSQSLVHSNGNTYLHWYLOKPGQSPKLLIYKV  
SNRFGVPPDRFSGSGGTDFTLKISRVEAEDLGVIYFCSTHVPYTFGGGTGLEIKHHHHHH

Figure 1

## D) anti-CD3 VHVL stL x 4-7 VHVL (SEQ ID NO: 13)

GATATCAAAC TGACAGT CAGGGGCTGAACTGGCAAGACCTGGGGCCTCAGTGAAGATGTCCTGCAAGACTTC  
TGGCTACACCTTTACTAGGTACACGATGCACTGGGTAAACACAGAGGCCCTGGACAGGCTCTGGAATGGATTGGAT  
ACATTAATCCTAGCCGTGGTTATACTAATTAACAATCAGAAGTTCAAGGACAAGGCCACATTGACTACAGACAAA  
TCCCTCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATA  
TTATGATGATCATTA TGCCTTGACTACTGGGGCCAAAGCACCACTCTCACAGTCTCCTCAGGTGGTGGTGGTT  
CTGGCGGGCGGCTCCGGTGGTGGTTCTGACATTCAGCTGACCCAGTCTCCAGCAATCATGCTGCATCT  
CCAGGGGAGAAGGTCACCATGACCTGCAGAGCCAGTTCAAGTGTAAGTTACATGAAC TGGTACCAGCAGAAGTC  
AGGCACCTCCCCAAAGATGGATTATGACACATCCAAAGTGGCTTCTGGAGTCCCTTATCGCTTCAGTGGCA  
GTGGGTCTGGGACCTCA TACTCTCTCACAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA  
CAGTGGAGTAGTAACCGCTCACGTTCCGGTGGTGGACCAAGCTGGAGCTGAAATCCGGAGGTGGTGGATCCGA  
GGTGCA GCTGCTCGAGCAGTCTGGAGCTGAGCTGGCGAGGCCCTGGGCTTCAGTGAAGCTGTCCCTGCAAGGCTT  
CTGGCTACACCTTCACAAACTATGGTTAAGCTGGTGAAGCAGAGGCCCTGGACAGGTCCTTGAGTGGATTGGA  
GAGGTTATCCTAGAAATTGGTAATGCTTACTACAATGAGAA GTTCAAGGGCAAGGCCACACTGACTGCAGACAA  
ATCCTCCAGCACAGGTC CATGGAGCTCCGCAGCCTGACCTCTGAGGACTCTGCGGTCTATTCTGTGCAAGAC  
GGGATCCTACGATACTAACTACGACTGGTACTTCGATGTCTGGGCCAAGGGACCAAGGTCACCGTCTCCTCA  
GGTGGTGGTGGTCTGGCGGGCGGCTCCGGTGGTGGTGGTCTGAGCTCGTGATGACCCAGACTCCACTCTC  
CCTGCCCTGT CAGTCTTGGAGATCAAGCCTCCATCTCTTG CAGATCTAGTCAGAGCCTTGTA CACAGTAATGGAA  
ACACCTATTACAT TGGTACCTGCAGAAAGCCAGGCCAGTCTCCAAGCTCCTGATCTACAAAGTTTCCAACCGA  
TTTTCTGGGGTCCCAGACAGGTT CAGTGGCAGTGGATCAGGGACAGATTTCCACAC

## Figure 1 D) continued

TCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGAGTTATTCTGTCTCTCAAAGTACACATGTTCCGTACACG  
TTCGGAGGGGGACCAAGCTTGAGATCAAACATCATCACCATCATCATAG

## (SEQ ID NO: 14)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGQLEWIGYINPSRGYTNYNQKFKDKATLTTDK  
SSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQSTTLTVSSGGGGGGGGGGSDIQLTQSPAIMSAS  
PGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPRFSGSGTSYSLTSSMEAEADAATYYCQ  
QWSSNPLTFGAGTKLELKS GGGSEVQLLEQSGAELARPGASVKLSCKASGYTFFTNYGLSWVKQRPQGQVLEWIG  
EVYPRIGNAYNEKFKGKATLTADKSSSTASMELRLSTSEDSAVYFCARRGSYDNTYDWYFDVWGQGTITVSS  
GGGSGGGGGGGSELVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLHWY LQKPGQSPKLLIYKVSNR  
FSGVPDRFSGSGGTDFTLKISRVEAEDLGVYFCSSQSTHVPYTFGGGGTKLEIKHHHHHH





## Figure 1 E) continued

AGGACTCTGCGGTCCTATTCTGTGCAAGACGGGGATCCTACGATACTAACTAGACTGGTACTTCGATGTCTGG  
GGCCAAGGACCACGGTCACCGTCTCCTCACATCATCACCATCATCATTAG

## (SEQ ID NO: 16)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKORPGQGLEWIGYINPSRGYTNYNQKFKDKATLTIDK  
SSSTAYMQLSSLTSEDSAVYVCARYDDHYCLDYWGQGTTLTVSSGGGGGGGGSDIQLTQSPAIMSAS  
PGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPIYRFSGSGTSYSLTISSEAEADAATYYCQ  
QWSSNPLTFGAGTKLELKSGGGSELVMTQTPLSLPVSLGDOASISCRSSQSLVHSNGNTYHLHWYLQKPGQSPK  
LLIYKVSNRFGVPDRFSGSGGTDFTLKISRVEAEDLGVIYFCSQSTHVPYTFGGGKLEIKGGGGSGGGSGG  
GGSEVQLLEQSGAELARPGASVKLSCKASGYTFITNYGLSWVKQRPQGVLEWIGEVYPRIGNAYYNEKFKGKATL  
TADKSSSTASMELRLTSEDSAVYFCARRGSDTNYDWFYFDVWGQGTITVTVSSHHHHH



Figure 1 F) continued

TCACCATCAGCAGTGTGCAGGCTGAAGACCTGGCAGTTATTACTGTCAAGATGATTATAGTTATCCGCTCACG  
TTCCGGTGCTGGGACCAAGCTTGAGATCAAACATCATGACCATCATCATTAG

(SEQ ID NO: 4)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGQGLEWIGYINPSRGYTNYNQKFKDKATLTDDK  
SSSTAYMQLSLTSSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSVEGGSGSGGVDIQLTQSPAIM  
SASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPRFSGSGSGTSYSLTSSMEAEADAATY  
YCQQWSSNPLTFGAGTKLELKS GGGSEVQLLEQSGAELVRPGTSVKISCKASGYAFNHWLGVVKQRPFGHGLE  
WIGDIFPGSGNIHYNEKFKGKATLTADKSSSTAYMQLSLTFEDSAVYFCARLRNWDPEMDYWGQGTTVTVSSG  
GGSGGGGGGSELVMTQSPSSITVTAGEKVTMSCKSSQSLNSGNQKNYLTWYQQKPGQPPKLLIYWASTR  
ESGVPDRFTGSGSGTDFTLTITSSVQAEDLAVYYCQNDYSYPLTFFGAGTKLEIKHHHHH

Figure 1

## G) anti-CD3 VHVL aL Ser x 5-10 VHVL (SEQ ID NO: 9)

GATATCAAACTGCAGCAGTCAGGGGCTGAACCTGGCAAGACCTGGGGCCTCAGTGAAGATGTCCTGCAAGACTTC  
TGGCTACACCTTTACTAGGTACACGATGCACCTGGGTAAACAGAGGCCCTGGACAGGGTCTGGAATGGATTGGAT  
ACATTAATCCTAGCCGTGGTTATACTAATTACAATCAGAAGTTCAAGGACAAGGCCACATTGACTACAGACAAA  
TCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATA  
TTATGATGATCATTAATCCCTTGACTACTGGGCCAAGGCACCACTCTCACAGTCTCCTCAGTCGAAGGTGGAA  
GTGGAGGTTCTGGTGGAAGTGGAGGTTCAAGTGGAGTCGACGACATTCAGCTGACCCAGTCTCCAGCAATCATG  
TCTGCATCTCCAGGGAGAAGGTCACCATGACCTGCAGAGCCAGTTCAAGTGTAAAGTTACATGAACCTGGTACCA  
GCAGAAAGTCAGGCACCTCCCCAAAAGATGGATTATGACACATCCAAAGTGGCTTCTGGAGTCCCCTTATCGCT  
TCAGTGGCAGTGGGCTCTGGGACCTCATACTCTCTCACAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTAT  
TACTGCCAAACAGTGGAGTAGTAACCCGCTCACGTTCCGTGGTGGACCAAGCTGGAGCTGAAATCCGGAGGTGG  
TGGATCCGAGGTGCAGCTGCTCGAGCAGTCTGGAGCTGAGCTGGTAAAGCCCTGGGACTTCAGTGAAGATATCCT  
GCAAGGCTTCTGGATACGCCCTTCACATACTAGCTAGGTTGGGTAAAGCAGAGGCCCTGGACATGGACTTGAG  
TGGATTGGAGATATTTCCCTGGAAGTGGTAATATCCACTACAATGAGAAAGTTCAAGGGCAAAAGCCACACTGAC  
TGCAGACAAATCTTCGAGCACAGCCTATATGCAGCTCAGTAGCCTGACATTTGAGGACTCTGCTGTCTATTCT  
GTGCAAGACTGAGGAACCTGGGACGAGCCTATGGACTACTGGGCCAAGGGACCAAGTCAACCGTCTCCTCAGGT  
GGTGGTGGTTCTGGCGGCGGCTCCGGTGGTGGTGGTCTGAGCTCGTGATGACACAGTCTCCATCCTCCCT  
GACTGTGACAGCAGGAGAGAAGGTCACATATGAGCTGCAAGTCCAGTCAGAGTCTGTTAAACAGTGGGAAATCAAA  
AGAACTACTTGACCTGGTACCAGCAGAAACCAAGGCAGCCTCCTAAACTGTTGATC

## Figure 1 G) continued

TACTGGGCATCCACTAGGGAATCTGGGGTCCCTGATCGCTTACAGGCAGTGGATCTGGAACAGATTTCACCTCT  
CACCATCAGCAGTGTGCAGGCTGAAGACCTGGCAGTTTATTACTGTCAGAATGATTATAGTTATCCGCTCACGT  
TCGGTGCTGGGACCAAGCTTGAGATCAAAACATCATCACCATCATCATAG

## (SEQ ID NO: 10)

DIKLQQSGAEIARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGQLEWIGYINPSRGYTNYNQKFKDKATLTDDK  
SSSTAYMQLSLTSSEDSAVYICARYDDHYSLDYWGQGTTLTVSSVEGGSGSGGVDIQLTQSPAIM  
SASPGKVTMTCRASSSVSVMNYYQQKSGTSPKRWIYDTSKVASGVYPYRFSGSGSTSYSLTISSEAEADAATY  
YCQWSSNPLTFGAGTKLELKSGGGSEVQLLEQSGAELVRPGTSVKISCKASGYAFTNYWLGWVKQRPQGHGLE  
WIGDIFPGSGNIHYNEKFKGKATLTADKSSSTAYMQLSLTFEDSAVYFCARLRNWDPEPMDYWGQGTTVTVSSG  
GGSGGGSGGGSELVMTQSPSSLTVTAGEKVTMSCKSSQSLNSGNQKNYLTWYQQKPGQPPKLLIYWASTR  
ESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCQNDYSYPLTFGAGTKLEIKHHHHH

Figure 1

## H) anti-CD3 VHVL stL x 5-10 VHVL (SEQ ID NO: 17)

GATATCAAACCTGCAGCAGTCAGGGGCTGAACTGGCAAGACCTGGGGCCTCAGTGAAGATGTCTCTGCAAGACTTC  
TGGCTACACCTTTACTAGGTACACGATGCACCTGGGTAAACACAGAGGCCCTGGACAGGCTCTGGAATGGATTGGAT  
ACATTAAATCCTAGCCGTGGTTATACTAATTACAATCAGAAAGTTCAAGGACAAGGCCACATTGACTACAGACAAA  
TCCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATA  
TTATGATGATCATTAATGCTTACTACTGCTGGGCCAAGGCACCACTCTCACAGTCTCCTCAGGTGGTGGTGGTT  
CTGGCGCGCGGCTCCGGTGGTGGTTCTGACATTCAGCTGACCCAGTCTCCAGCAATCATGTCTGCATCT  
CCAGGGGAGAAGGTCACCATGACCTGCAGAGCCAGTTCAAGTGTAAGTTACATGAACCTGGTACCAGCAGAAGTC  
AGGCACCTCCCCAAAAGATGGATTATGACACATCCAAAGTGGCTTCTGGAGTCCCTTATCGCTTCAGTGGCA  
GTGGTCTGGGACCTCATACTCTCACAAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA  
CAGTGGAGTAGTAACCGCTCACGTTCCGGTGTGGTGGACCAAGCTGGAGCTGAAATCCGGAGGTGGTGGATCCGA  
GGTGAGCTGCTCGAGCAGTCTGGAGCTGAGCTGGTAAGGCCCTGGGACTTCAGTGAAGATATCCTGCAAGGCTT  
CTGGATACGCCCTTCACTAACTACTGGCTAGGTTGGTAAAGCAGAGGCCCTGGACATGGACTTGAGTGGATTGGA  
GATATTTCCCTGGAAGTGGTAATATCCACTACAATGAGAAAGTTCAAGGGCAAAGCCACACTGACTGCAGACAA  
ATCTTCGAGCACAGCCTATATGCAGCTCAGTAGCCTGACATTTGAGGACTCTGCTGTCTATTCTGTGCAAGAC  
TGAGGAACTGGGACGAGCCTATGGACTACTGGGGCCAAAGGACCAACGGTCAACCGTCTCCTCAGGTGGTGGTGGT  
TCTGGCGCGGCTCCGGTGGTGGTTCTGAGCTCGTGATGACACAGTCTCCATCCTCCCTGACTGTGAC  
AGCAGGAGAGAAGGTCACTATGAGCTGCAAGTCCAGTCAGAGTCTGTAAACAGTGGAAATCAAAAGAACTACT  
TGACCTGGTACCAAGCAGAAACCAAGGCAGCCTCCTAAACTGTTGATCTACTGGGCATCCACTAGGGAATCTGGG  
GTCCCTGATCGCTTCAAGGCAGTGGATCTGGAACAGATTTCACTCTCACCATCA

## Figure 1 H) continued

GCAGTGTGCAGGCTGAAGACCTGGCAGTTTATTACTGTCAGAATGATTATAGTATCCGCTCACGTTTCGGTGCT  
GGGACCAAGCTTGAGATCAAACATCATCACCATCATCATTAG

## (SEQ ID NO: 18)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGLEWIGYINPSRGYTNYNQFKDKATLTDDK  
SSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSGGGGGGGGSDIQLTQSPAIMSAS  
PGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVYPYRFSGSGGTSYSLTISSEAEADAATYYCQ  
QWSSNPLTFGAGTKLELKSGGGSEVQLLEQSGAELVRPGTSVKISCKASGYAFTNHWLGVKQRPFGHGLEWIG  
DIFPGSGNIHYNEKFKGKATLTADKSSSTAYMQLSSLTFEDSAVYFCARLRNWDPEMDYWGQGTTLTVSSGGGG  
SGGGSGGGSELVMTQSPSSLTVTAGEKVTMSCKSSQSLNSGNQKNYLTWYQQKPGQPPKLLIYWASTRESG  
VPDRFTGSGSGTDFTLTISSVQAEDLAVYYCQNDYSYPLTFGAGTKLEIKHHHHHH



Figure 1

## D) anti-CD3 VHVL stL x 5-10 VL VH (SEQ ID NO: 19)

GATATCAAAC TGACGAGT CAGGGCTGA ACTGGCAAGACCTGGG CCTCAGTGAAGATGTCCTGCAAGACTTC  
TGGCTACACCTTTACTAGGTACACGATGCAC TGGTAAACAGAGG CCTGGACAGGCTCTGGAATGGATTGGAT  
ACATTAATCCTAGCCGTTATATACTAATTACAATCAGAA GTTCAAGGACAAGGCCACATTGACTACAGACAAA  
TCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGA CATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATA  
TTATGATGATCATTA CTGCC TTGACTACTG GGGCC AAGCCACTCTCAGAGTCTCCTCAGGTGGTGGTGGTT  
CTGGCGGGCGGCTCCGGTGGTGGTTCTGACATT CAGCTGACCCAGTCTCCAGCAATCATGTCTGCATCT  
CCAGGGAGAAGGTCA CCA TGACCTGCAGAGCCAGTTC AAGTGTAA GTTACATGA ACTGGTACCAGCAGAAAGTC  
AGGCACCTCCCCAAAAGATGGATTATGACACATCGAAA GTGGCTTCTGGAGTCCCTTATCGCTTCAGTGGCA  
GTGGTCTGGGACCTCATACTCTCTCACAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA  
CAGTGGAGTAGTAACCGCTCACGTTCCGGTCTGGGACCAAGCTGGAGCTGAAATCCGGAGGTGGTGGATCCGA  
GCTCGTGATGACACAGTCTCCATCCTCCCTGACTGTGACAGCAGGAGAGAAGTCACTATGAGCTGCAAGTCCA  
GTCAGAGTCTGTAAACAGTGGAAATCAAAGA AACTACTTGACCTGGTACCAGCAAGAAC CAGGCGAGCCTCCT  
AAACTGTTGATCTACTGGGCATCCACTAGGGAATCTGGG GTCCCTGATCGCTTCA CAGGCAGTGGATCTGGAAC  
AGATTTCACCTCTCACCATCAGCAGTGTGCAGGCTGAAGACCTGGCAGTTTATTACTGT CAGAA TGATTATAGTT  
ATCCGCTCACGTTCCGGTCTGGACCAAGCTTGAGATCAAAGGTGGTGGTGGTTCTGGCGGGCGGCTCCGGT  
GGTGGTGGTTCTGAGGTGCAGTGTCTCGAGCAGTCTGGAGCTGAGCTGGTAAGCCTGGGACTTCAGTGAAGAT  
ATCCTGCAAGGCTTCTGGATACGCCCTTCACTAACTACTGGCTAGGTTGGGTAAAGCAGAGGCCCTGGACATGGAC  
TTGAGTGGATTGGAGATATTTCCCTGGAGTGGTAAATATCCACTACAATGAGAA GTTCAAGGGCAAAGCCACA  
CTGACTGCAGACAAAATCTTCGAGCACAGCCCTATATG CAGCTCAGTAGCCTGACAT

## Figure 1 I) continued

TTGAGGACTCTGCTCTATTCTGTGCAAGACTGAGGAAGCTGGGACGAGCCTATGGACTACTGGGGGCCCAAGGG  
ACCACGGTCACCGTCTCCTCACATCATCACCATCATCATTAG

## (SEQ ID NO: 20)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTIDK  
SSSTAYMQLSSLTSEDSAVYICARYYDDHYCLDYWGQGTTLTVSSGGGGGGGGSDIQLTQSPAIMASAS  
PGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPRFSGSGTSYSLTISMEAEADAATYYCQ  
QWSSNPILTFGAGTKLELKSGGGSELVMTQSPSSLTVTAGEKVTMSCKSSQSLNSGNQKNYLTWYQQKPGQPP  
KLLIYWASTRESGVPRFTGSGSGTDFTLTISVQAEDLAVYYCQNDYSYPLTFGAGTKLEIKGGGGSGGGSG  
GGSEVQLLEQSGAELVRPGETSVKISCKASGYAFTNFWLGVKQRPGLHWIGDIFPGSGNIHYNEKFKGKAT  
LTADKSSSTAYMQLSSLTFEDSAVYFCARLRNWDEPMDYWGQGTTVTVSSHHHHHH

Figure 1

## J) anti-CD3 VHVL aL x 3-1 VHVL (SEQ ID NO: 45)

GATATCAAACCTGCAGCAGTCAGGGGCTGAACCTGGCAAGACCTGGGGCCTCAGTGAAGATGTCCTGCAAGACTTC  
TGGCTACACCTTTACTAGGTACACGATGCACCTGGGTAAACACAGAGGCCCTGGACAGGGTCTGGAATGGATTGGAT  
ACATTAATCCCTAGCCGTGTTATACTAATTAACAATCAGAAGTTCAAGGACAAGGCCACATTGACTACAGACAAA  
TCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATA  
TTATGATGATCATTACTGCCCTTGACTACTGGGGCCCAAGGCCACCTCTCACAGTCTCCTCAGTCGGAAGTGGAA  
GTGGAGGTTCTGGTGAAGTGAGGTTCAGGTGGAGTCGACGACATTCAGCTGACCCAGTCTCCAGCAATCATG  
TCTGCAATCTCCAGGGAGAAGTCAACCATGACCTGCAGAGCCAGTTCAAGTGTAAGTTACATGAACCTGGTACCA  
GCAGAACTCAGGCACCTCCCCCAAAGATGGATTTATGACACATCCAAAAGTGGCTTCTGGAGTCCCCTTATCGCT  
TCAGTGGCAGTGGTCTGGACCTCATACTCTCTCACAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTAT  
TACTGCCAACAGTGGAGTAGTAACCCGCTCACGTTCCGGTGGTGGACCAAGCTGGAGCTGAAATCCGGAGGTGG  
TGGATCCGAGGTGCAGCTGCTCGAGCAGTCTGGAGCTGAGCTGGTGAACCTGGGGCCTCAGTGAAGATATCCT  
GCAAGGCTTCTGGATACGCCCTTCACTAACTACTGGCTAGGTTGGGTAAGCAGAGGCCCTGGACATGGACTTGAG  
TGGATTGGAGATCTTTTCCCTGGAAGTGGTAATACTCACTACAATGAGAGGTTCAGGGGCAAGCCACACTGAC  
TGCAGACAAATCCTCGAGCACAGCCTTTATGCAGCTCAGTAGCCTGACATCTGAGGACTCTGCTGTCTATTCT  
GTGCAAGATTGAGGAACCTGGACGAGGCTATGGACTACTGGGGCCCAAGGGACCAAGTCAAGTCTCCTCAGGT  
GGTGGTGGTTCTGGCGGGGGGCTCCGGTGGTGGTCTGAGCTCGTCATGACCCAGTCTCCATCTTATCT  
TGCTGCATCTCCTGGAGAAACCATTAATAATTGCAGGGCAAGTAAGAGCATTAGCAATAATTAGCCTGGT  
ATCAAGAGAAACCTGGGAAAACATAAAGCTTCTTATCTACTCTGGATCCACTTTGCAATCTGGAATTCATCA  
AGGTTCAGTGGCAGTGGATCTGGTACAGATTTCACTCTCACCATCAGTAGCCCTGG

## Figure 1 J) continued

AGCCTGAAGATTTTGCAATGTATTACTGTCAACAGCATTAATGAATATCCGTACACGTTCCGAGGGGGACCAAG  
CTTGAGATCAAAACATCATCACCATCATCATTAG

## (SEQ ID NO: 46)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGQLEWIGYINPSRGYTNYNQKFKDKATLITDK  
SSSTAYMQLSSLTSEDSAVYYCARYDDHYCLDYWGQGTTLTVSSVEGGSGGSGGVDDIQLTQSPAIM  
SASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPRFSGSGTSYSLTISSMEAEDAATY  
YCQQWSSNPLTFGAGTKLELKSGGGGSEVQLLEQSGAELVKPGASVKISCKASGYAFITNYWLGWVKQRPQGHGLE  
WIGDLFPGSGNTHYNERFRGKATLTADKSSSTAFMQLSSLTSEDSAVYFCARLRNWDAMDYWGQGTITVTVSSG  
GGSGGGGGGGSELVMTQSPSYLAASPGETITINCRASKSISKYLAWYQEKPKTNKLLIYSGSTLQSGIPS  
RFGSGSGTDFTLTISGLEPEDFAMYYCQQHNEYPTTFGGGTKLEIKHHHHH.

Figure 1

K) anti-CD3 VHVL aL Ser x 3-1 VHVL (SEQ ID NO: 47)

GATATCAAACCTGCAGCAGTCAAGGGGCTGAACTGGCAAGACCTGGGGCCCTCAGTGAAGATGTCTCTGCAAGACTTC  
 TGGCTACACCTTTACTAGGTACACGATGCACTGGGTAAACACAGAGGCCCTGGACAGGGTCTGGAATGGATTGGAT  
 ACATTAATCCTAGCCGTGGTTATACTAATTACAATCAGAAGTTCAAGGACAAGGCCACATTGACTACAGACAAA  
 TCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATA  
 TTATGATGATCATTAATCCCTTGACTACTGGGCCAAGGCACCACTCTCACAGTCTCCCTCAGTCGAAGGTGGAA  
 GTGGAGGTTCTGGTGGAAAGTGGAGGTTCAAGGTGGAGTCGACGACATTCAGCTGACCCAGTCTCCAGCAATCATG  
 TCTGCATCTCCAGGGGAGAAGGTCACCATGACCTGCAGAGCCAGTTCAAAGTGTAAGTTACATGAAGTGGTACCA  
 GCAGAAAGTCAGGCACCTCCCCCAAAGATGGATTTATGACACATCCAAAGTGGCTTCTGGAGTCCCTTATCGCT  
 TCAGTGGCAGTGGTCTGGGACCTCATACTCTCTCACAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTAT  
 TACTGCCAACAGTGGAGTAGTAACCCGCTCACGTTCCGGTGTGGGACCAAGCTGGAGCTGAAATCCGGAGGTGG  
 TGGATCCGAGGTGCACTGCTCGAGCAGTCTGGAGCTGAGCTGGTGAAACCTGGGGCCCTCAGTGAAGATAATCCT  
 GCAAGGCTTCTGGATACGGCTTCACTAACTACTGGCTAGGTTGGGTAAAGCAGAGGCCCTGGACATGGACTTGAG  
 TGGATTGGAGATCTTTCCCTGGAAAGTGGTAATACTCACTACAATGAGAGGTTCAGGGGCAAAAGCCACACTGAC  
 TGCAGACAAATCCCTCGAGCACAGCCTTTATGCAAGCTCAGTAGCCTGACATCTGAGGACTCTGCTGTCTATTCT  
 GTGCAAGATTGAGGAACCTGGGACGAGGCTATGGACTACTGGGGCCAAAGGACCACGGTCACCGTCTCCTCAGGT  
 GGTGGTGGTTCTGGCGGGCGGCTCCGGTGGTGGTCTGAGCTCGTCAATGACCCAGTCTCCATCTTATCT  
 TGTGCACTCTCCTGGAGAAACCATTAATAATTGCAGGGCAAGTAAGACATTAGCAAATAATTAGCCTGGT  
 ATCAAGAGAAACCTGGGAAAACATAAAGCTTCTTATCTACTCTGGATCCACTTTG

## Figure 1 K) continued

CAATCTGGAATCCATCAAGGTTCA GTGGCAGTGGATCTGGTACAGATTTCAC TCTCACCATCAGTAGCCTGGA  
GCCTGAAGATTTTGCAATGTATTACTGTCAACAGCATAAATGAATATCCGTACACGTT CGGAGGGGGACCAAGC  
TTGAGATCAAACATCATCACCATCATCATTAG

## (SEQ ID NO: 48)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGLEWIGYINPSRGYTNYNQKFKDKATLLTTDK  
SSSTAYMQLSSLTSEDSAVYVCARYYDDHYSLDYWGQGTTLTVSSVEGGSGGSGGVDDIQLTQSPAIM  
SASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISSMEAEADAATY  
YQQWSSNPLTFGAGTKLELKSGGGSEVQLLEQSGAELVKPGASVKISCKASGYAFTNYWLGVVKQRPFGHGLE  
WIGDLFPGSGNTHYNERFRGKATLTADKSSSTA FMOQLSSLTSEDSAVYFCARLRNWD EAMDYWGQGTTVTVSSG  
GGSGGGGGGGSELVMTQSPSYLAASPGETITINCRASKSISKYLA WYQEKPGKTNKLLIYSGSTLQSGIPS  
RFSGSGSGTDFTLTISLLEPEDFAMYYCQQHNEYPYTFGGG TKLEIKHHHHHH

Figure 1

## L) anti-CD3 VHVL aL x 3-5 VHVL (SEQ ID NO: 49)

GATATCAAACCTGCAGCAGTCAAGGGCTGAACCTGGCAAGACCTGGGGCTCAGTGAAGATGTCCTGCAAGACTTC  
TGGCTACACCTTTACTAGGTACACGATGCACTGGGTAAACACAGAGGCCCTGGACAGGGTCTGGAATGGATTGGAT  
ACATTAATCCTAGCCGTGTTATACTAATTACAATCAGAAGTTCAAGGACAAGGCCACATTGACTACAGACAAA  
TCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATA  
TTATGATGATCATTTACTGCCTTGACTACTGGGGCCAAAGCCACCACTCTCACAGTCTCCTCAGTCCAAGGTGGAA  
GTGGAGGTTCTGGTGGAGTGGAGGTTCAAGTGGAGTCGACGACATTCAGCTGACCCAGTCTCCAGCAATCATG  
TCTGCATCTCCAGGGAGAAAGGTCAACATGACCTGCAGAGCCAGTTCAAGTGAAGTTACATGAACCTGGTACCA  
GCAGAACTCAGGCACCTCCCCAAAAGATGGATTTATGACACATCCAAAAGTGGCTTCTGGAGTCCCTTATCGCT  
TCAGTGGCAGTGGGTC TGGACCTCATACTCTCTCACAAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTAT  
TACTGCCAACAGTGGAGTAGTAACCCGCTCACGTTCCGGTGGTGGACCAAGCTGGAGCTGAAATCCGGAGGTGG  
TGGATCCGAGGTGCAGCTGCTCGAGCAGTCTGGAGCTGAGCTGGTAAGGCCCTGGGACTTCAGTGAAGCTGTCT  
GCAAGGCTTCTGGCTACACCTTCACAAGCTATGGTTTAAAGCTGGGTGAAGCAGAGAACTGGACAGGGCCTTGAG  
TGGATTGGAGAGGTTTATCCTAGAAATTGGTAATGCTTACTACAATGAGAAGTTCAGGGCAAGGCCACACTGAC  
TGCAGACAAATCCTCCAGCACAGCGTCCATGGAGCTCCGCAGCCTGACATCTGAGGACTCTGCGGTCATTCT  
GTGCAAGACGGGATCCTACGGTAGTAACTACGACTGGTACTTCGATGCTCTGGGGCCAAAGGCCACCGTCAAC  
GTCTCCTCAGGTGGTGGTTCTGGCGGGCGGGCTCCGGTGGTGGTTCTGAGCTCGTGAATGACCCAGAC  
TCCACTCTCCCTGTCAGTCTTGGAGATCAAGCCTCCATCTCTTGCAGATCTAGTCAGAGCCTTGTACACA  
GTAATGGAAACACCTATTTACATTTGGTACCTGCAGAAAGCCAGGCCAGTCTCCAAAAGCTCCTGATCTACAAAGTT  
TCCAAACCGATTTTCTGGGGTCCCAGACACAGGTTCACTGGCAGTGGATCAGGGACAG

Figure 1 L) continued

ATTTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTATTCTGCTCTCAAAGTACACATGTT  
CCGTACACGTTCCGAGGGGGACCAAGCTTGAGATCAAAACATCATCACCATCATTAG

(SEQ ID NO: 50)

DIKIQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDK  
SSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSVEGGSGSGGVDDIQLTQSPAIM  
SASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISSEAEADAATY  
YCQQWSSNPLTFAGATKLELKS GGGSEVQLLEQSGAELVRPGTSVKLSCKASGYTFTSYGLSWVKQRTGQGLE  
WIGEVYPRIGNAYYNEKFKGKATLTADKSSSTASMELRLTSEDSAVYFCARRGSYGSNYDWYFDVWGQGTVT  
VSSGGGGSGGGGGSELVMTQTPLSLPVSLGDAQASISCRSSQSLVHSNGNTYLHWYLQKPGQSPKLLIYKV  
SNRFSGVPRDFSGSGGTDFTLKISRVEAEDLGVIYFCSSQSTHVPYTFGGGKLEIKHHHHH



Figure 1

## M) anti-CD3 VHVL aL Ser x 3-5 VHVL (SEQ ID NO: 51)

GATATCAAACTGCAGCAGTCAGGGCTGAACTGGCAAGACCTGGGGCCTCAGTGAAGATGTCTGCAAGACTTC  
TGGCTACACCTTTACTAGGTACACGATGCACCTGGGTAAACAGAGGCCCTGGACAGGGTCTGGAATGGATTGGAT  
ACATTAAATCCTAGCCGTGTTATACTAATTACAATCAGAAGTTCAAGGACAAGGCCACATTGACTACAGACAAA  
TCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATA  
TTATGATGATCATTAATCTCCCTTGACTACTGGGGCCCAAGCACCACTCTCACAGTCTCCTCAGTCGAAGGTGGAA  
GTGGAGGTTCTGGTGGAGTGAGGTTCAAGTGGAGTCGACGACATTCAGCTGACCCAGTCTCCAGCAATCATG  
TCTGCATCTCCAGGGAGAAAGTCAACATGACCTGCAGAGCCAGTTCAAGTGTAAGTTACATGAACCTGGTACCA  
GCAGAAGTCAGGCACCTCCCCAAAAGATGGATTTATGACACATCCAAAGTGGCTTCTGGAGTCCCCTTATCGCT  
TCAGTGGCAGTGGGCTCTGGACCTCATACTCTCTCACAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTAT  
TACTGCCAACAGTGGAGTAGTAACCCGCTCACGTTCCGGTGGTGGACCAAGCTGGAGCTGAAATCCGGAGGTGG  
TGGATCCGAGGTGCAGCTGCTCGAGCAGTCTGGAGCTGAGCTGGTAAGGCCCTGGGACTTCAGTGAAGCTGTCTCT  
GCAAGGCTTCTGGCTACACCTTCACAAGCTATGGTTTAAGCTGGTGAGCAGAGAACTGGACAGGGCCTTGAG  
TGGATTGGAGAGGTTTATCCTAGAAATTGGTAATGCTTACTACAATGAGAAAGTTCAAGGGCAAGGCCACACTGAC  
TGCAGACAAATCCTCCAGCACAGCGTCCATGGAGCTCCGCAGCCTGACATCTGAGGACTCTGCGGTCATTCTCT  
GTGCAAGACGGGGATCCTACGGTAGTAACCTACGACTGGTACTTCCGATGTCTGGGGCCAAAGGGACCCACGGTCACC  
GTCCTCCTCAGGTGGTGGTTCTGGCGGGCGGGCTCCGGTGGTGGTTCTGAGCTCGTGATGACCCAGAC  
TCCACTCTCCCTGCTCAGTCTTGGAGATCAAGCCTCCATCTCTTGAGATCTAGTCAGAGCCTTGTAACACA  
GTAATGGAAACACCTATTACATTGGTACCTGCAGAAAGCCAGGCCAGTCTCCAAAGCTCCTGATCTACAAAGTT  
TCCAAACCGATTCTCTGGGTCCCAGACAGGTTCAAGTGGCAGTGGATCAGGGACAG

## Figure 1 M) continued

ATTTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTTATTCTGCTCTCAAAGTACACATGTT  
CCGTACACGTTCCGAGGGGGACCAAGCTTGAGATCAAACATCATCACCATCATCATTAG

(SEQ ID NO: 52)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGQGLEWIGYINPSRGYTNYNQKFKDKATLTDDK  
SSSTAYMQLSSLTSEDSAVYICARYYDDHYSLDYWGQGTTLTVSSVEGGSGGSGGVDDIQLTQSPAIM  
SASPGEKVMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPIYRFSGSGSGTSYSLTISSMEEADAATY  
YCQQWSSNPLTTFGAGTKLELKS GGGSEVQLLEQSGAELVRPGTSVKLSCKASGYTFTSYGLSWVKQRTGQGLE  
WIGEVYPRIGNAYYNEKFKGKATLTADKSSSTASMEIRSLTSEDSAVYFCARRGSYGSNYDWYFDVWGQGTIVT  
VSSGGGGSGGGGGSELVMTQTPLSLPVSIGDQASISCRSSQSLVHSNGNTYLVHWYLOKPGQSPKLLIYKV  
SNRFSGVDPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSSQSTHVPYTFGGGTGLEIKHHHHHH

Figure 1

N) anti-CD3 VHVL stL x 3-5 VHVL (SEQ ID NO: 53)

GATATCAAACTGCAGCAGTCAGGGGCTGAACCTGGCAAGACCTGGGGCCTCAGTGAAGATGTCCTGCAAGACTTC  
 TGGCTACACCTTTACTAGGTACACGATGCACCTGGGTAAACACAGAGGCCCTGGACAGGGTCTGGAATGGATTGGAT  
 ACATTAAATCCTAGCCGTGGTTATACTAATACAATCAGAACTCAAGGACAAGGCCACATTGACTACAGACAAA  
 TCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATA  
 TTATGATGATCATTAATGCTTGAATACTGGGGCCAAAGGACCACTCTCACAGTCTCCTCAGGTGGTGGTGGTT  
 CTGGCGGGCGGCTCCGGTGGTGGTTCTGACATTCAGCTGACCCAGTCTCCAGCAATCATGTCTGCATCT  
 CCAGGGGAGAAAGTCAACATGACCTGCAGGCCAGTTCAAGTGTAAAGTTACATGAACCTGGTACCAGCAGAAAGTC  
 AGGCACCTCCCCAAAGATGGATTATGACACATCCAAAGTGGCTTCTGGAGTCCCTTATCGCTTCAGTGGCA  
 GTGGGTCTGGGAOCTCATACTCTCACAAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA  
 CAGTGGAGTAGTAAACCGCTCACGTTCCGGTGTGGGACCAAGCTGGAGCTGAAATCCGGAGGTGGTGGATCCGA  
 GGTGCAGCTGCTCGAGCAGTCTGGAGCTGAGCTGGTAAGGCTGGGACTTCAGTGAAGCTGTCTCCTGCAAGGCTT  
 CTGGCTACACCTTCACAAGCTATGGTTTAAAGCTGGTGAAGCAGAGAACTGGACAGGGCCTTGAGTGGATTGGA  
 GAGGTTTATCCTAGAAATTGGTAATGCTTACTACAATGAGAACTCAAGGGCAAGGCCACACTGACTGCAGACAA  
 ATCCTCCAGCACAGCGTCCATGGAGCTCCGAGCCTGACATCTGAGGACTCTGCGGTCTATTCTGTGCAAGAC  
 GGGATCCTACGGTAGTAACTACGACTGGTACTTCGATGTCTGGGGCCAAAGGACCACCGTCAACCGTCTCCTCA  
 GTGGTGGTGGTTCTGGCGGGCGGCTCCGGTGGTGGTGGTCTGAGCTCGTGATGACCCAGACTCCACTCTC  
 CCTGCCCTGTGAGTCTTGAGATCAAGCCTCCATCTCTTGAGATCTAGTCAGAGCCTTGTAACACAGTAATGGAA  
 ACACCTATTACATTGGTACCTGCAGAAAGCCAGGCCAGTCTCCAAAGCTCCTGATCTACAAAAGTTTCCAAACCGA  
 TTTTCTGGGGTCCCAGACAGGTTCAAGTGGCAGTGGATCAGGGACAGATTTCACAC

## Figure 1 N) continued

TCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTTATTCTGTCTCTCAAAGTACACATGTTCCGTACACG  
TTCGGAGGGGGACCAAGCTTGAGATCAAACATCATCACCATCATCATTAG

## (SEQ ID NO: 54)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGQGLEWIGYINPARGYTNYNQKFKDKATLLTTDK  
SSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTLLTVSSGGGGGGGGGGSDIQLTQSPAIMSAS  
PGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVYRFSGSGSGTSYSLTSSMEAEADAATYYCQ  
QWSSNPLTFGAGTKLELKSGGGGSEVQLLEQSGAELVRPGTSVKLSCKASGYTFTSYGLSWVKQRTGQGLEWIG  
EVYPRIGNAYYNEKFKGKATLTADKSSSTASMELRSLTSEDSAVYFCARRGSYGSNYDWYFDVWGQGTITVTVSS  
GGGSGGGGGGSELVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLLHWYLOKPGQSPKLLLIYKVSNR  
FSGVPDRFSGSGGTDFTLKISRVEAEDLGVYFCSQSTHVPYTFGGGTKLEIKHHHHH

Figure 1

O) anti-CD3 VHVLaL x 4-1 VHVLaL (SEQ ID NO: 55)

GATATCAAACATGCAGCAGTCAAGGGCTGAACCTGGCAAGACCTGGGGCTCAGTGAAGATGTCTGTCAAGACTTC  
TGGCTACACCTTTACTAGGTACACGATGCATGGGTAAACACAGAGGCTTGACAGGGTCTGGAATGGATTGGAT  
ACATTAACTCTAGCCGTGTTACTAATAATACAATCAGAAGTTCAAGGACAAGGCCACATTGACTACAGACAAA  
TCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATA  
TTATGATGATCATTAATGCTTGACTACTGGGGCCAAAGGCACACTCTCACAGTCTCTCAGTCTGAAAGGTGGAA  
GTGAGGTTCTGGTGGAAGTGGAGTTCAAGTGGAGTCGACGACATTCAGCTGACCCAGTCTCCAGCAATCATG  
TCTGCACTCTCAGGGGAGAAGTCAACATGACCTGCAGAGCCAGTTCAAAGTGAAGTTACATGAACCTGGTACCA  
GCAGAAATCAGGCACCTCCCCAAAAGATGGATTATGACACATCCAAAGTGCTCTGGAGTCCCTTATCGCT  
TCAGTGGCAGTGGGTCTGGACCTCATACTCTCACAAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTAT  
TACTGCCAACAGTGGAGTAGTAACCCGCTCACGTTCCGTCCTGGGACCAAGCTGGAGCTGAAATCCGGAGGTGG  
TGGATCCGAGGTGCAGCTGCTCGAGCAGTCTGGAGCTGAGCTGGTAAGGCCCTGGGACTTCAGTGAAGATATCCT  
GCAAGGCTTCTGGATACGCCCTTCACTAACTACTGGCTAGGTTGGTTAAGCAGAGGCCCTGGACATGGACTTGAA  
TGGGTTGGAGATATTTCCCTGGAAGTGGTAATGCTCACTACAATGAGAAATTCAAGGGCAAAAGCCACACTGAC  
TGACAGACAAAGTCCCTGTACACAGCCTATATGCAGCTCAGTAGCCTGACATCTGAGGACTCTGCTGTCTATTTCT  
GTGCAAGATTGCGGAACTGGGACGAGGCTATGGACTACTGGGGCCAAAGGCCACCGTCAACCGTCTCCTCAGGT  
GGTGGTGGTCTTGGCGGGGGCTCCGGTGGTGGTCTGAGCTCGTGATGACACAGTCTCCATCCTCCCT  
GAGTGTGTACGAGGAGAAAGTCACATATGAGCTGCAAGTCCAGTCAGAGTCTGTTAAACAGTGGAATCAAA  
AGAACTACTTGGCTTGTACCAAGCAAAACAGGGCAGCCTCCTAAACTGTTGATCTACGGGGCATCCACTAGG  
GAATCTGGGGTCCCCTGATCGCTTCACAGGCAGTGGATCTGGAACACAGATTTCACTC

## Figure 1 O) continued

TCACCATCAGCAGTGTGCAGGCTGAAGACCTGGCAGTTATTACTGTCAAGATGATTATAGTTATCCGTACACG  
TTCGGAGGGGGACCAAGCTTGAGATCAAAACATCATCACCATCATCATTAG

## (SEQ ID NO: 56)

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDK  
SSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSVEGGSGGSGGVDIQLTQSPAIM  
SASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIMDTSKVASGVPYRFSGSGSGTSYSLTISMEAEADAATY  
YCQQWSSNPLTFGAGTKLELKSGGGGSEVQLLEQSGAELVRPGTSVKISCKASGYAFTNYYLWLVKQRPQGHGLE  
WVGDIFFPGSGNAHYNEKFKGKATLTADKSSYTAYMQLSSLTSEDSAVYFCARLRNWDYWGQGTTVTVSSG  
GGSGGGGGGSELVMTQSPSSLSVSAGEKVTMSCKSSQSLNLSGNQKNYLAWYQQKPGQPPKLLIYGASTR  
ESGVPDRFTGSGSGTDFTLTITISSVQAEDLAVYYCQNDYSYPYTFGGGGTKLEIKHHHHHH

Figure 1

P) anti-CD3 VHVL aL Ser x 4-1 VHVL (SEQ ID NO: 57)

GATATCAAACCTGCAGCAGTCAGGGGCTGAACTGGCAAGACCTGGGGCCTCAGTGAAGATGTCCTGCAAGACTTC  
TGGCTACACCTTTACTAGGTACACGATGCACCTGGGTAAACACAGAGGCCCTGGACAGGGTCTGGAATGGATTGGAT  
ACATTAATCCTAGCCGTGGTTATATACTAATTACAATCAGAAAGTTCAAGGACAAGGCCACATTGACTACAGACAAA  
TCCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATA  
TTATGATGATCATTAATCTCCCTTGACTACTGGGGCCAAAGCCACCACTCTCACAGTCTCCTCAGTCCGAAGGTGGAA  
GTGGAGGTTCTGGTGGAAAGTGGAGGTTCAGGTGGAGTCGACGACATTCAGCTGACCCAGTCTCCAGCAATCATG  
TCTGCATCTCCAGGGGAGAAAGTCAACCATGACCTGCAGAGCCAGTTCAAGTGTAAAGTTACATGAACCTGGTACCA  
GCAGAAAGTCAGGCACCTCCCCCAAAAGATGGATTATGACACATCCAAAGTGGCTTCTGGAGTCCCTTATCGCT  
TCAGTGGCAGTGGTCTGGGACCTCATACTCTCTCAAAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTAT  
TACTGCCAAACAGTGGAGTAGTAACCCGCTCACGTTCCGGTGTCTGGGACCAAGCTGGAGCTGAAATCCGGAGGTGG  
TGGATCCGAGGTGCAGCTGCTCGAGCAGTCTGGAGCTGAGCTGGTAAGGCCCTGGGACTTCAGTGAAGATATCCT  
GCAAGGCTTCTGGATACGCCCTTCACTAACTACTGGCTAGGTTGGTTAAGCAGAGGCCCTGGACATGGACTTGAA  
TGGGTTGGAGATATTTCCCTGGAAAGTGGTAATGCTCACTACAATGAGAAAGTTCAAGGGCAAGCCACACTGAC  
TGCAGACAAGTCCTCGTACACAGCCCTATATGCAGCTCAGTAGCCTGACATCTGAGGACTCTGCTGTCTATTCT  
GTGCAAGATTGCCGAACTGGGACGAGGCTATGGACTACTGGGGCCAAAGGACCACCGTCACTCCTCAGGT  
GGTGGTGGTTCTGGCGGGCGGCTCCGGTGGTGGTCTGAGCTCGTGATGACACAGTCTCCATCCTCCCT  
GAGTGTGTACAGCAGGAGAAAGGTCACTATGAGCTGCAAGTCCAGTCAGAGTCTGTAAACAGTGGAAATCAAA  
AGAACTACTTGGCCTGGTACCAGCAGAAACCAGGGCAGCCTCCTAACTGTTGATCTACGGGGCATCCACTAGG  
GAATCTGGGGTCCCTGATCGCTTCACAGGCAGTGGATCTGGAACAGATTTCACTC

## Figure 1 P) continued

TCACCATCAGCAGTGTGCAGGCTGAAGACCTGGCAGTTTATTACTGTGAGAAATGATTATAGTTATCCGTACACG  
TTCGGAGGGGGACCAAGCTTGAGATCAAACATCATCACCATCATCATTAG

## (SEQ ID NO: 58)

DIKLOQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPQGQLEWIGYINPSRGYTNYNQKFKDKATLLTTDK  
SSSTAYMQLSSLTSEDSAVYYCARYYDDHYSLDYWGQGTTLTVSSVEGGSGGSGGVDDIQLTQSPAIM  
SASPGGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPRFSGSGSGTSYSLTISSEAEADAATY  
YCQQWSSNPLTFGAGTKLELKSGGGGSEVQLLEQSGAEIVRPGETSVKISCKASGYAFTNYWLGWVKQRPQGHGLE  
WVGDIFFPGSGNAHYNEKFKGKATLTADKSSYTA YMQLSSLTSEDSAVYFCARLRNWD EAMD YWGQGT TVTVSSG  
GGSGGGGGGSELVMTQSPSSLSVSAGEKVTMSCKSSQSLNSGNQKNYLAWYQQKPGQPPKLLIYGASTR  
ESGVPPDRFTGSGSGTDFTLTISSVQAEDLAVYYCQNDYSYPYTFEGGGTKLEIKHHHHHH



Figure 1

## Q) anti-CD3 VHVL stL x 4-1 VHVL (SEQ ID NO: 59)

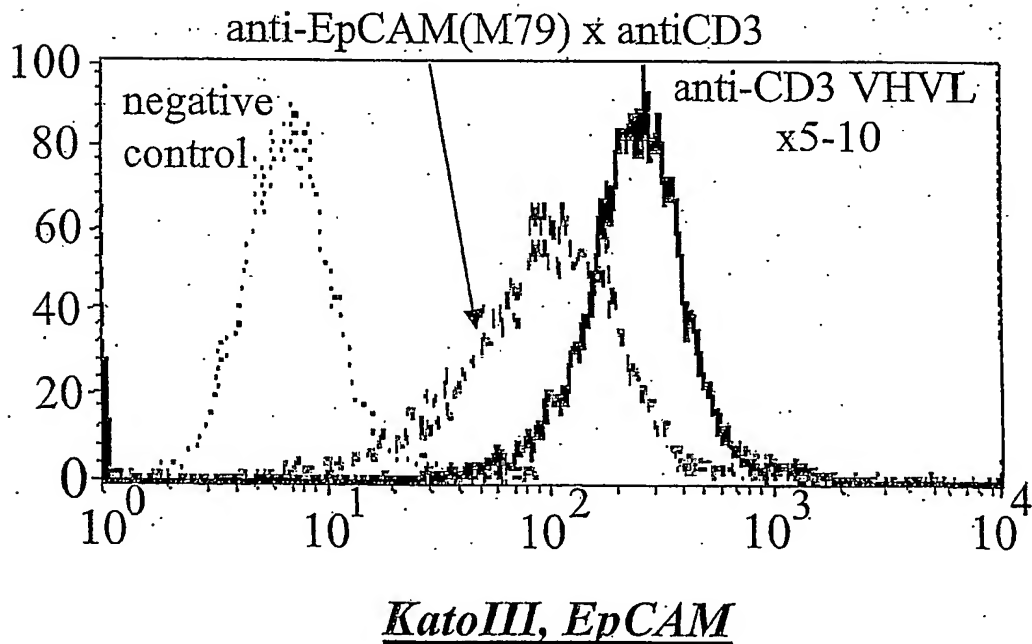
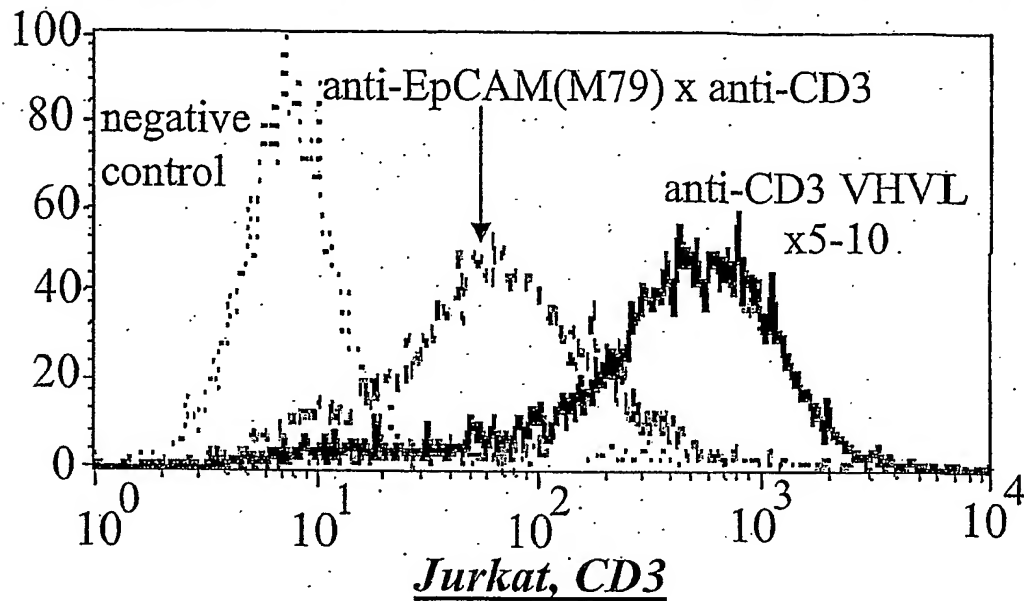
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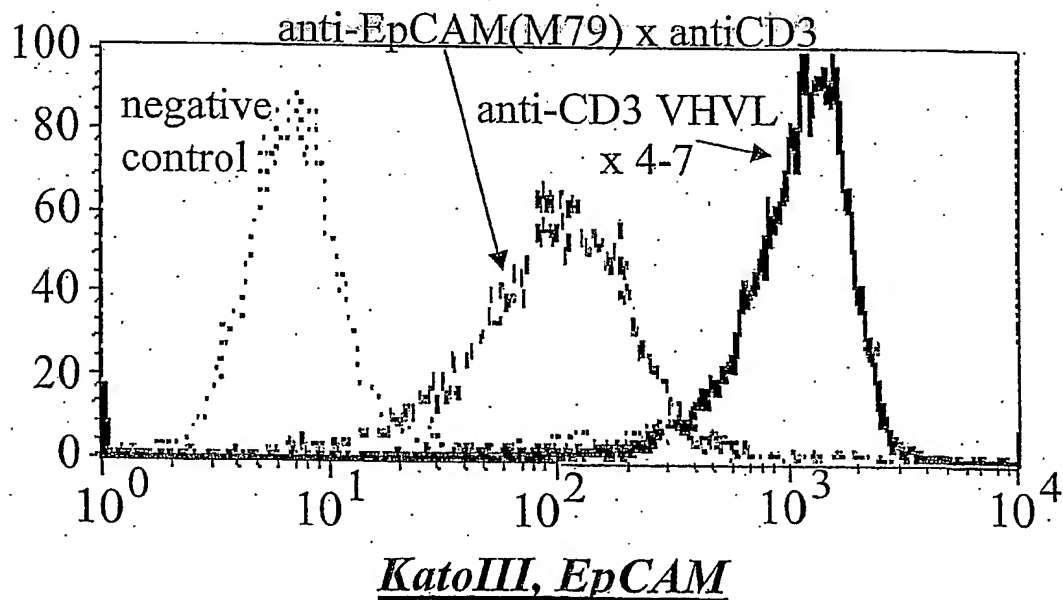
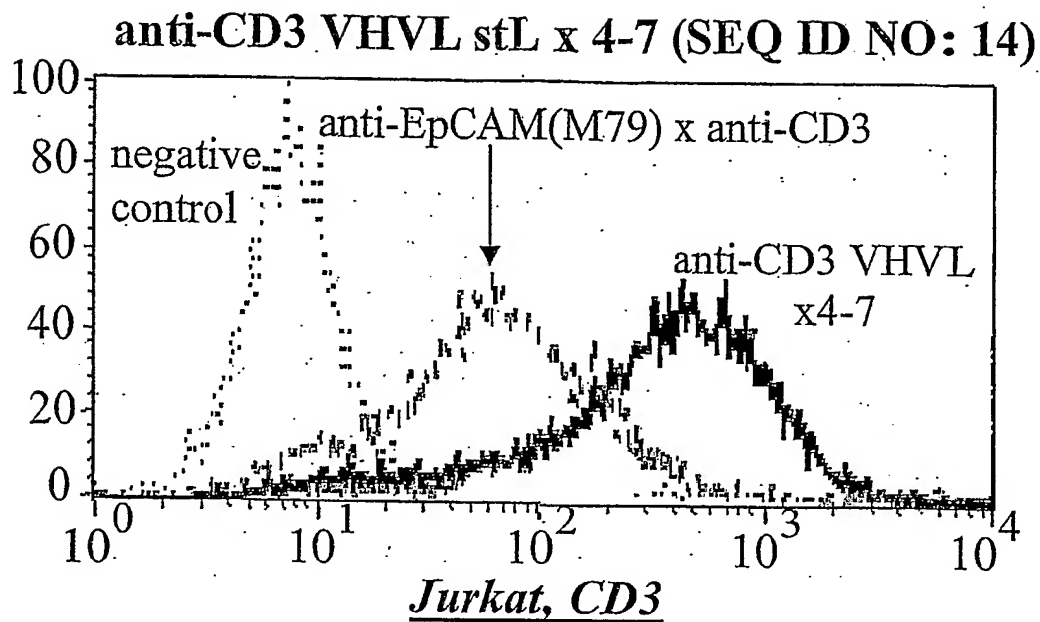
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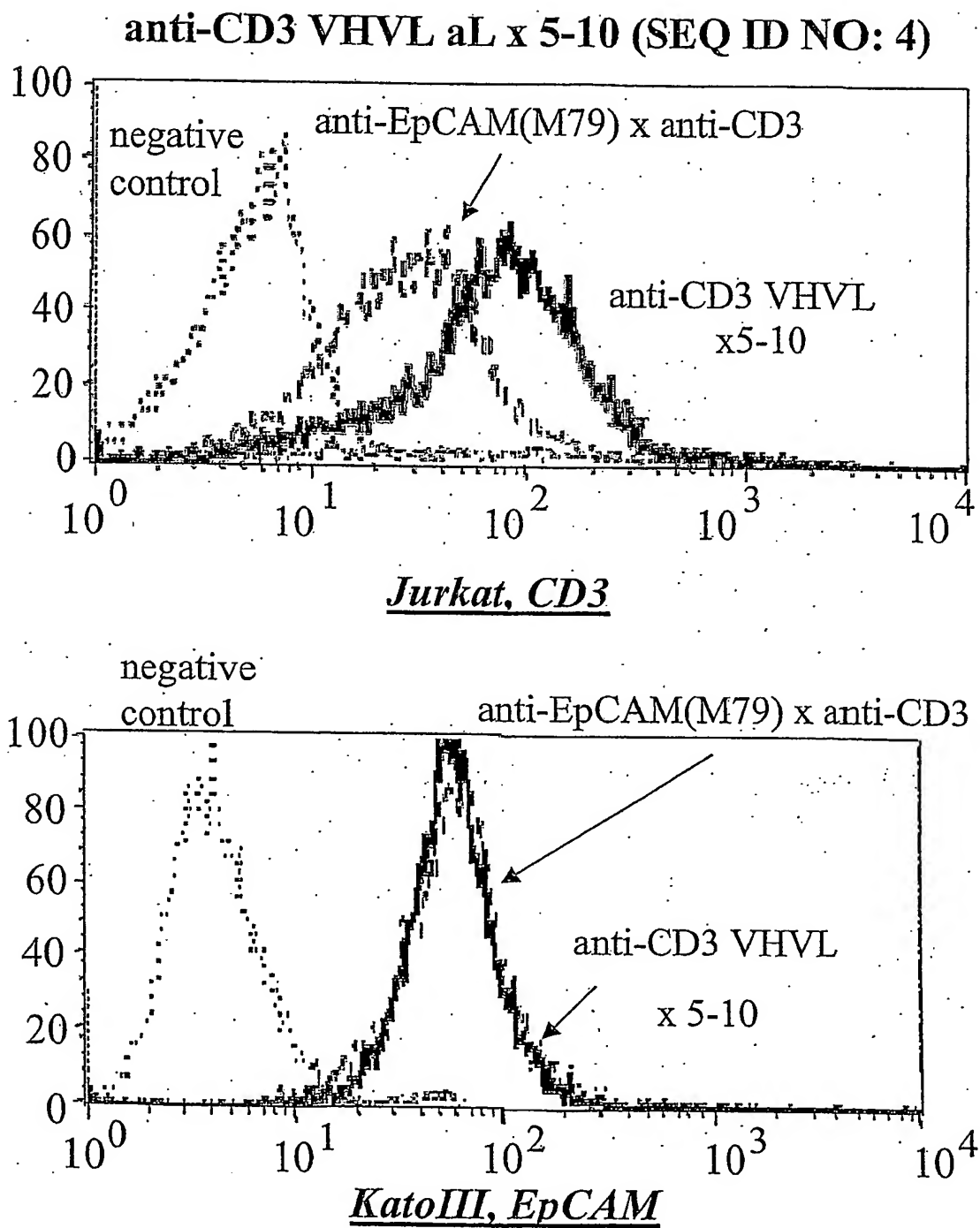
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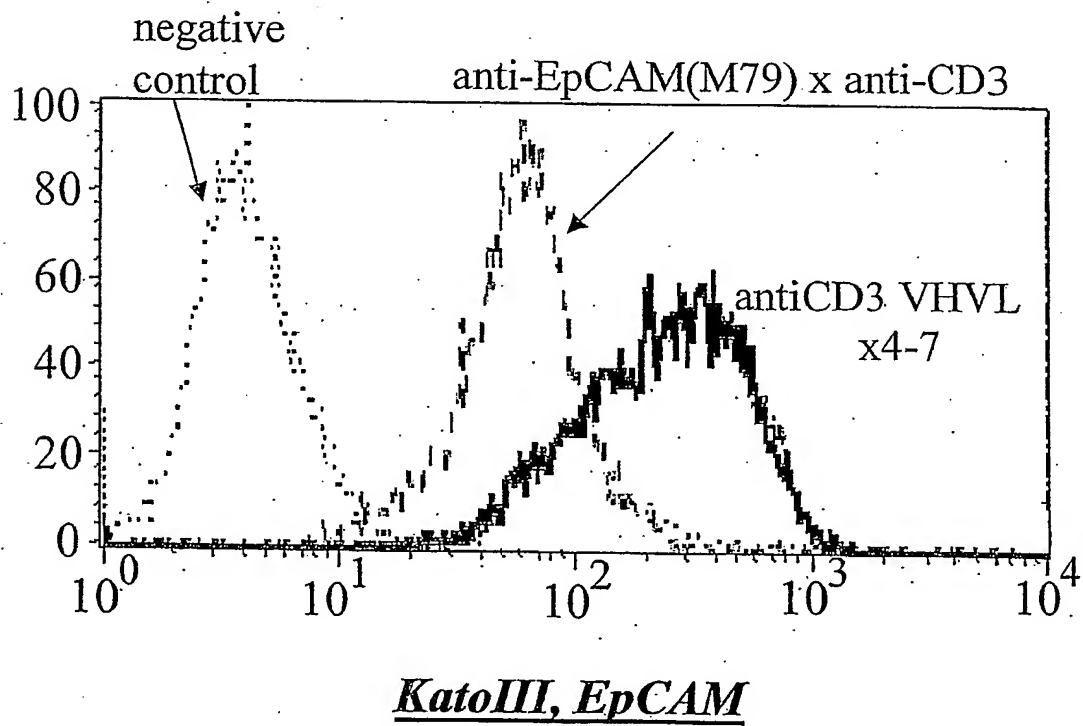
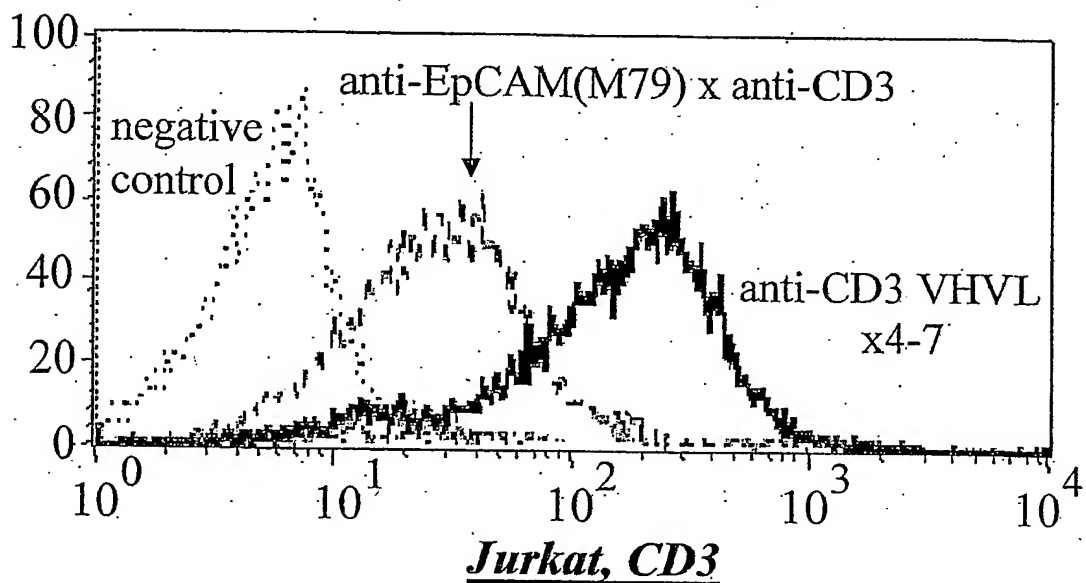
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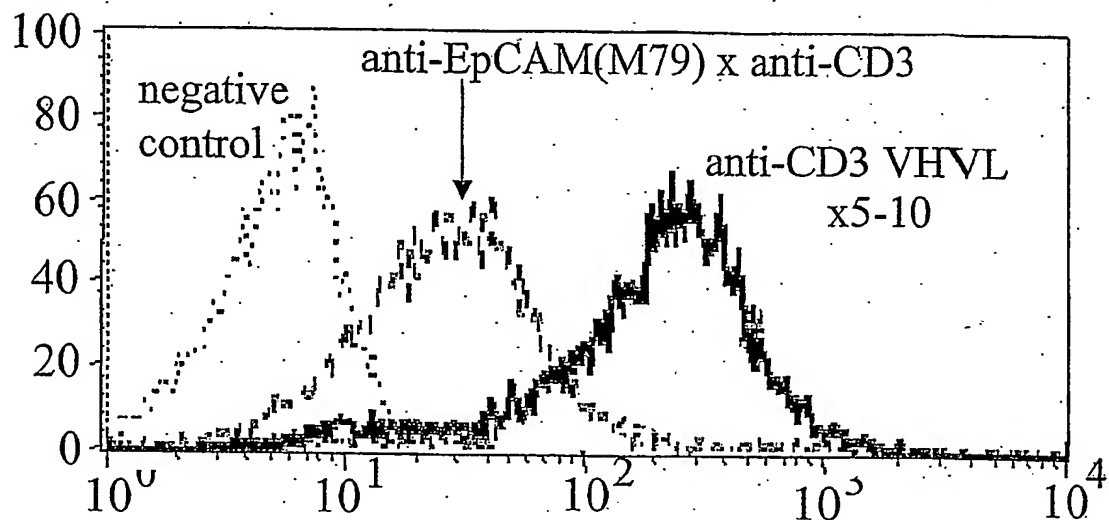
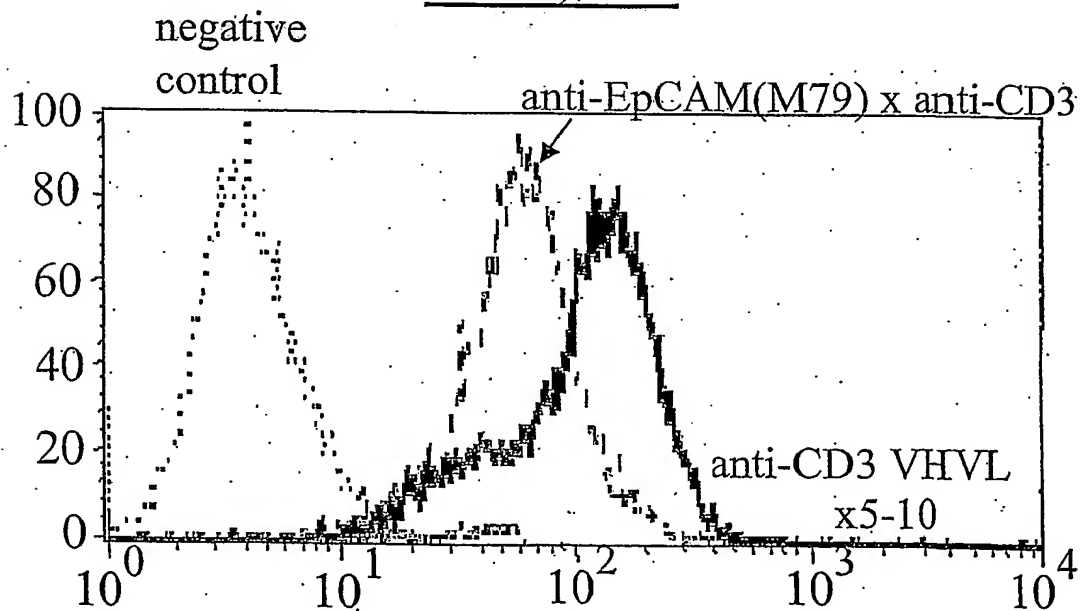
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QWSSNPLTFGAGTKLELKSGGGGSEVQLLEQSGAELVRPGTSVKISCKASGYAFTNYYLWLVKQRPQGHGLEWVG  
DIFPGSGNAHYNEKFKGKATLTADKSSYTAQMQLSSLTSEDSAVYFCARLRNWDYWGQGTTLTVSSGGGG  
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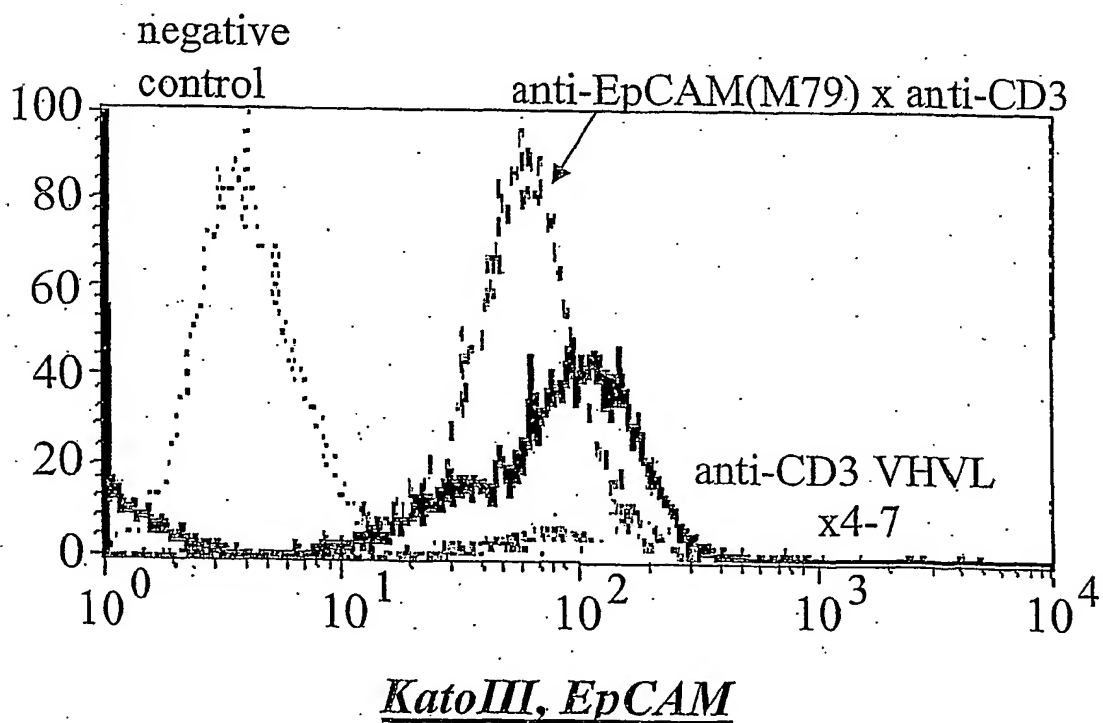
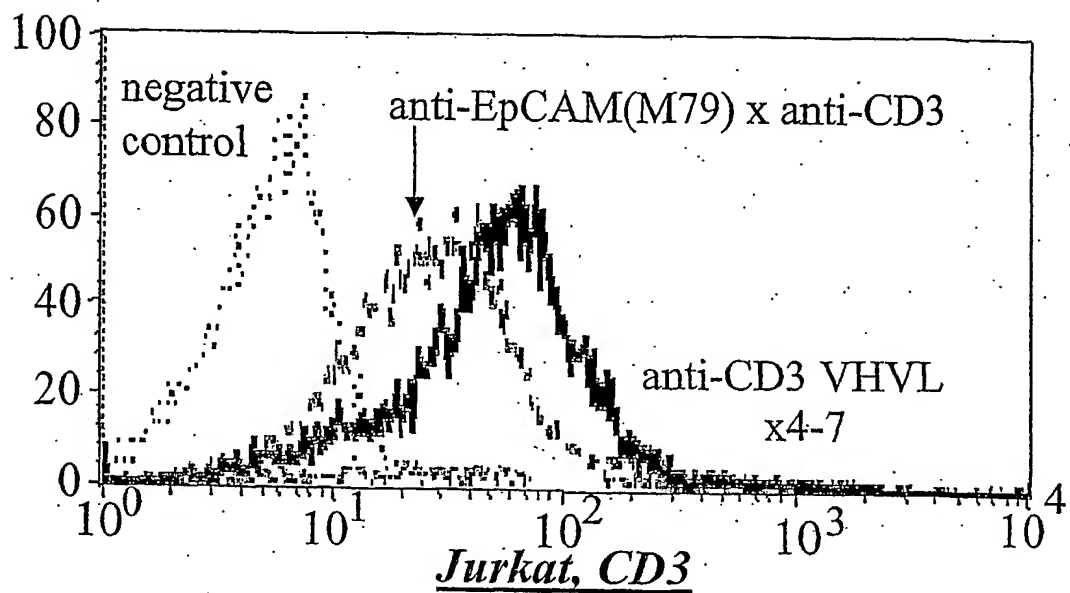
**Figure 2 A****anti-CD3 VHVL stL x 5-10 (SEQ ID NO:18)**

**Figure 2 B**

**Figure 2C**

**Figure 2D****anti-CD3 VHVL aL x 4-7 (SEQ ID NO: 2)**

**Figure 2E****anti-CD3VHVL aL Ser x 5-10 (SEQ ID NO: 10)****Jurkat, CD3****KatoIII, EpCAM**

**Figure 2F****anti-CD3 VHVL aL Ser x 4-7 (SEQ ID NO: 8)**



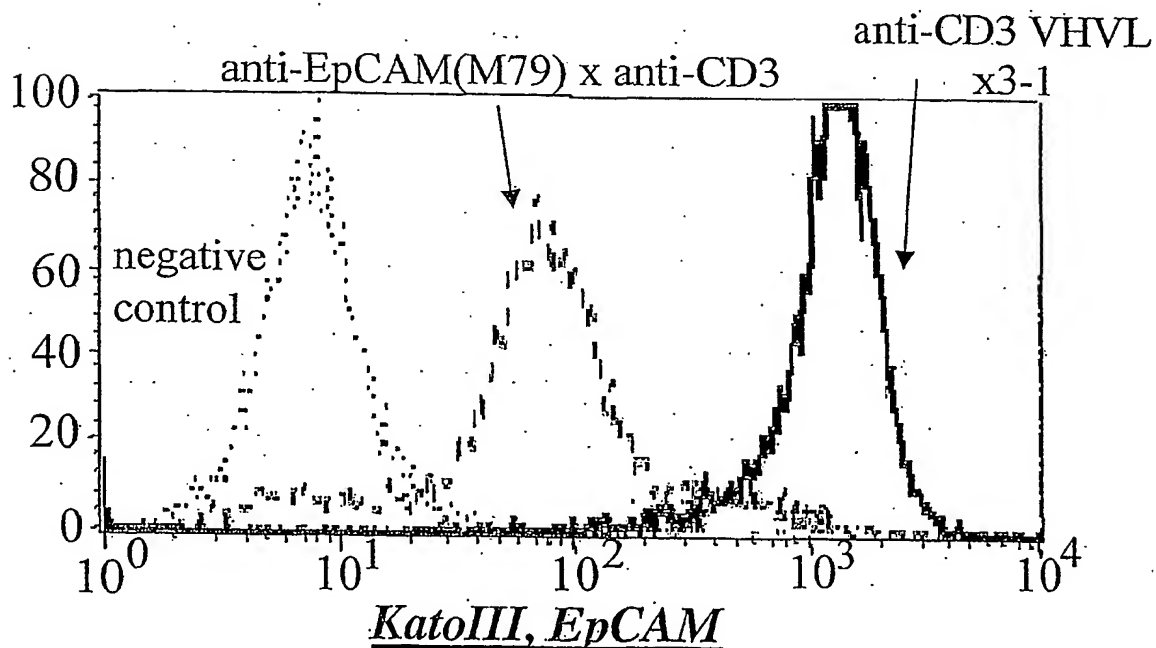
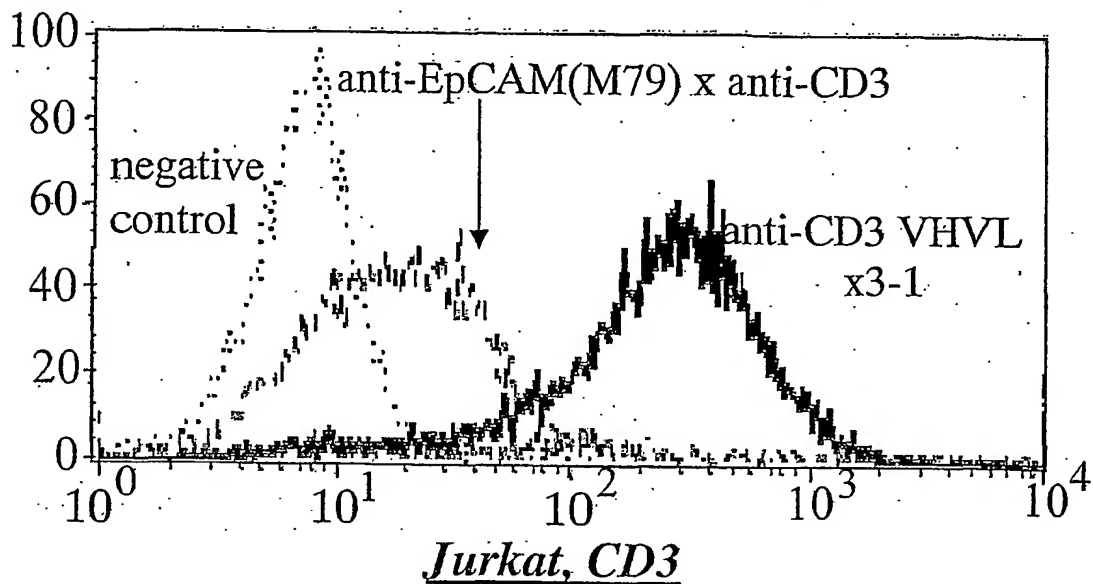
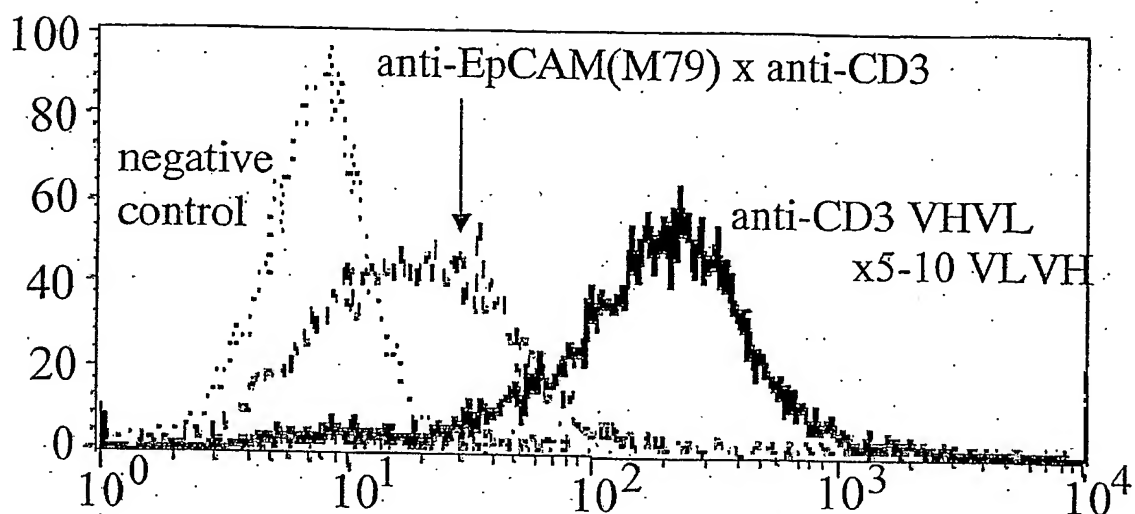
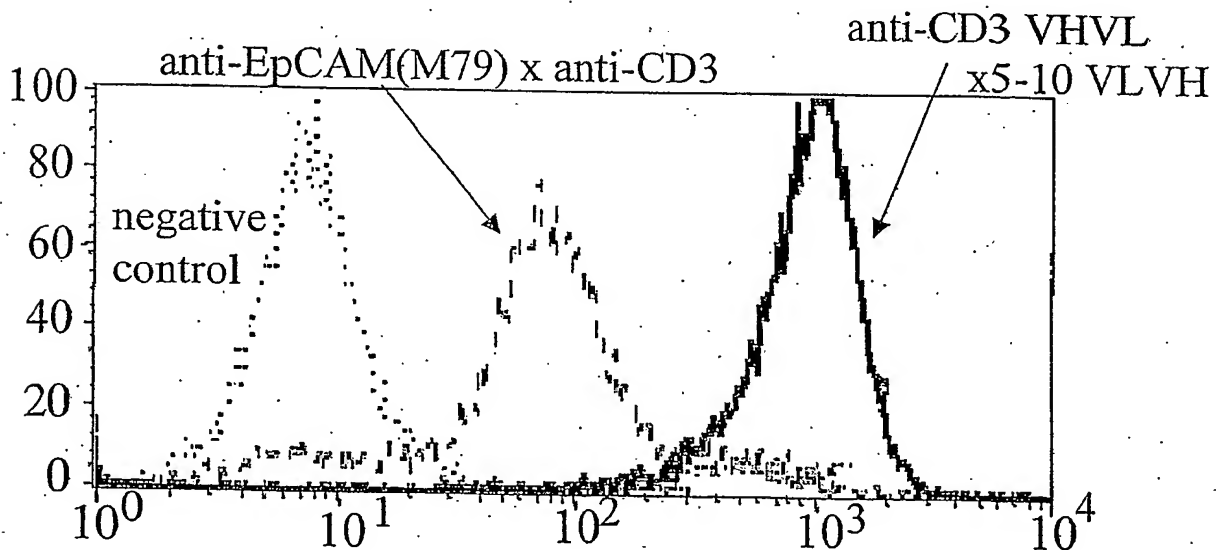
**Figure 2G****anti-CD3 VHVL stL x 3-1 (SEQ ID NO: 12)**

Figure 2H

**anti-CD3 VHVL stL x 5-10 VLVH (SEQ ID NO: 20)**



Jurkat, CD3



KatoIII, EpCAM

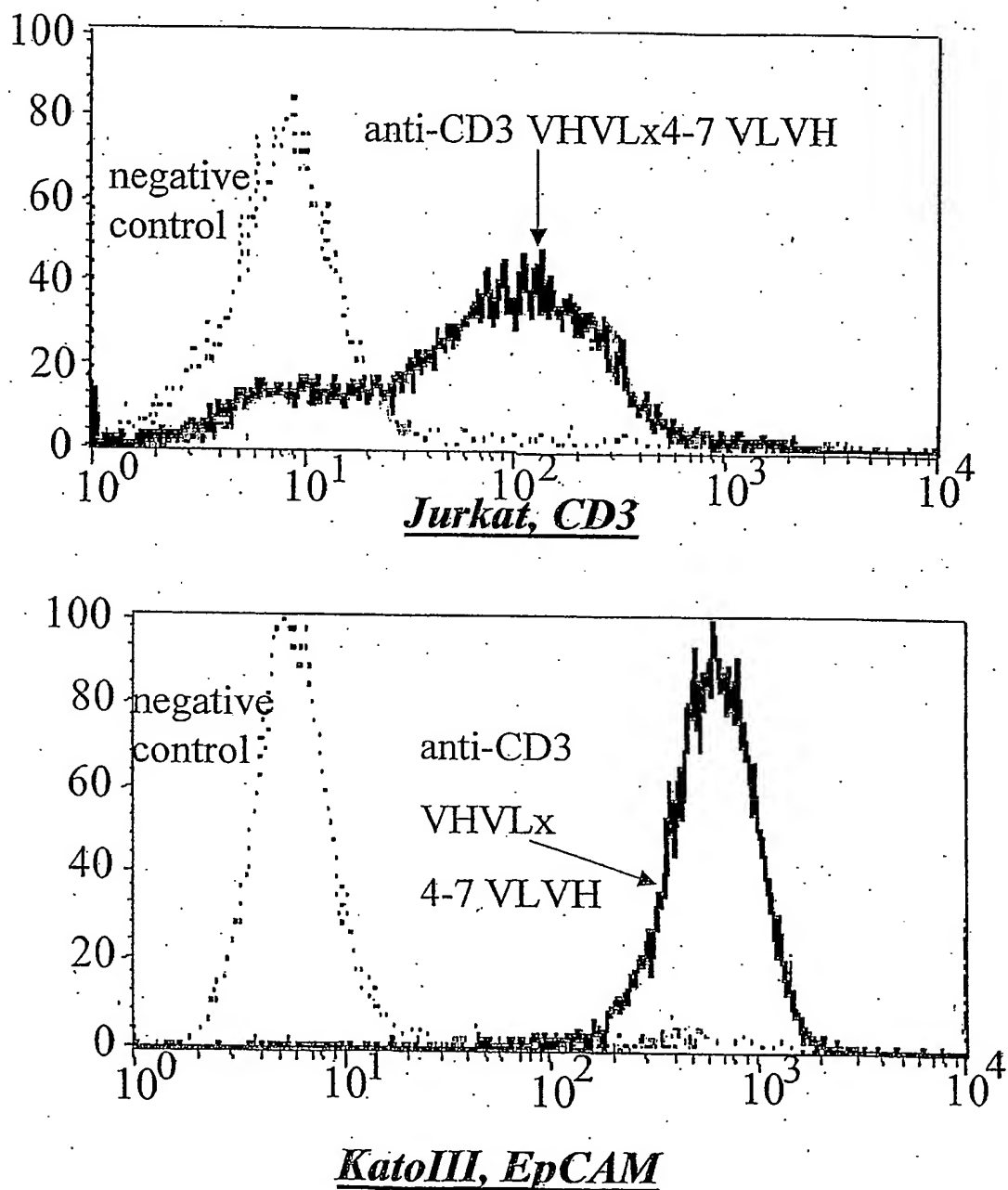
**Figure 2I****anti-CD3 VHVL stL x 4-7 VLVH (SEQ ID NO: 16)**

Figure 3A

## 4-7 (vLvH) x anti-CD3 (SEQ ID NO: 42)

1 MGWSCIIILFL VATATGVHSA RELVMTQTPL SLPVSLGDQA SISCRSSQSL  
51 VHSNGNTYLH WYLQKPGQSP KLLIYKVSNR FSGVPDRFSG SSGTDFTLK  
101 ISRVEAEDLG VYFCSQSTHV PYTFGGGTKL EIKGGGGSGG GSGGGGSEV  
151 QLLEQSGAEL ARPGASVKLS CKASGYTFN YGLSWVKQRP GQVLEWIGEV  
201 YPRIGNAYN EKFKGKATLT ADKSSSTASM ELRSLTSEDS AVYFCARRGS  
251 YDTNYDWYED VWGQGTTVTV SSGGGGSDIK LQSGAELAR PGASVKMSCK  
301 TSGYTFTRYT MHWVKQRPQG GLEWIGYINP SRGYTNYNQK FKDKATLTDD  
351 KSSSTAYMQL SSLTSEDSAV YYCARYYDDH YCLDYWGQGT TLTVSSVEGG  
401 SGGSGGSGGS GGVDDIQLTQ SPAIMSASPG EKVTMTCRAS SSVSYMWNWYQ  
451 QKSGTSPKRW IYDTSKVASG VPYRFSGSGS GTSYSLTISS MEAEDAATYY  
501 CQQWSSNPLT FGAGTKLELK HHHHHH\*

## Figure 3A (continued)

SEQ ID NO: 41

```
1  ATGGGATGGA  GCTGTATCAT  CCTCTTCTTG  GTAGCAACAG  CTACAGGTGT
51  AACTCCGG  CGGAGCTCG  TGATGACCCA  GACTCCACTC  TCCCTGCCCTG
101 TCAGTCTTGG  AGATCAAGCC  TCCATCTCTT  GCAGATCTAG  TCAGAGCCCTT
151 GTACACAGTA  ATGGAACAC  CTATTACAT  TGGTACCTGC  AGAAGCCAGG
201 CCAGTCTCCA  AAGTCCCTGA  TCTACAAAGT  TTCCAACCGA  TTTTCTGGGG
251 TCCCAGACAG  GTTCAGTGGC  AGTGGATCAG  GGACAGATTT  CACACTCAAG
301 ATCAGCAGAG  TGGAGGCTGA  GGATCTGGGA  GTTTATTTCT  GCTCTCAAAG
351 TACACATGTT  CCGTACACGT  TCGAGGGGG  GACCAAGCTT  GAGATCAAAG
401 GTGGTGGTGG  TTCTGGCGGC  GCGGCTCCG  GTGGTGGTGG  TTCTGAGGTG
451 CAGTGCTCG  AGCAGTCTGG  AGCTGAGCTG  GCGAGGCCCTG  GGCCTTCAGT
501 GAAGCTGTCC  TGCAAGGCTT  CTGGCTACAC  CTTCACAAAC  TATGGTTTAA
551 GCTGGGTGAA  GCAGAGGCCT  GGACAGGTCC  TTGAGTGGAT  TGGAGAGGTT
601 TATCCTAGAA  TTGGTAATGC  TTAATAAAT  GAGAAATTCA  AGGCAAGGC
651 CACACTGACT  GCAGACAAAT  CCTCCAGCAC  AGCGTCCATG  GAGCTCCGCA
701 GCCTGACCTC  TGAGGACTCT  GCGGTCTATT  TCTGTGCAAG  ACGGGATCC
751 TACGATACTA  ACTACGACTG  GTACTTCGAT  GTCTGGGGCC  AAGGGACCAC
801 GGTACCGTC  TCCTCCGGAG  GTGGTGGATC  CGATATCAAA  CTGCAGCAGT
851 CAGGGGCTGA  ACTGGCAAGA  CCTGGGGCCT  CAGTGAAGAT  GTCCTGCAAG
```

**Figure 3A (continued)**

```
901 ACTTCTGGCT ACACCTTTAC TAGGTACACG ATGCACCTGGG TAAACACAGAG
951 GCCTGGACAG GGTCTGGAAT GGATTGGATA CATTAATCCTT AGCCGTGGTT
1001 ATACTAATTA CAATCAGAAG TTCAAGGACA AGGCCACATT GACTACAGAC
1051 AAATCCTCCA GCACAGCCTA CATGCAACTG AGCAGCCTGA CATCTGAGGA
1101 CTCTGCAGTC TATTACTGTG CAAGATAATTA TGATGATCAT TACTGCCTTG
1151 ACTACTGGG CCAAGGCACC ACTCTCACAG TCTCCTCAGT CGAAGGTGGA
1201 AGTGGAGGTT CTGGTGGAAG TGGAGGTTCA GGTGGAGTCG ACGACATTCA
1251 GCTGACCCAG TCTCCAGCAA TCATGTCGC ATCTCCAGGG GAGAAGGTCA
1301 CCATGACCTG CAGAGCCAGT TCAAGTGTA GTTACATGAA CTGGTACCAG
1351 CAGAAAGTCAG GCACCTCCCC CAAAGATGG ATTTATGACA CATCCAAGT
1401 GGCTTCTGGA GTCCCTTATC GCTTCAGTGG CAGTGGGTCT GGGACCTCAT
1451 ACTCTCTCAC AATCAGCAGC ATGGAGGCTG AAGATGCTGC CACTTATTAC
1501 TGCCAACAGT GGAGTAGTAA CCCGCTCACG TTCGGTGCTG GGACCAAGCT
1551 GGAGCTGAAA CATCATCACC ATCATCATTA G
```

## Figure 3B

3-5 (vLvH) x anti-CD3 (SEQ ID NO: 30)

```
1  MGWSCIIILFL VATATGVHSA RELVMTQTPL SLPVSLGDQA SISCSSQSL
51  VHSNGNTYLH WYLQPGQSP KLLIYKVSNR FSGVPDRFSG SSGTDFTLK
101 ISRVEAEDLG VYFCSQSTHV PYTFGGGTKL EIKGGGGSGG GSGGGGSEV
151 QLEQSGAEL VRPGTSVKLS CKASGYTFTS YGLSWVKQRT GQLEWIGEV
201 YPRIGNAYYN EKFKGKATLT ADKSSSTASM ELRSLTSEDS AVYFCARRGS
251 YGSNYDWYFD VWGQGTTVTV SSGGGGSDIK LQSGAELAR PGASVKMSCK
301 TSGYTFTRYT MHWVKQRPQG GLEWIGYINP SRGYTNYNQK FKDKATLTID
351 KSSSTAYMQL SSLTSEDSAV YYCARYYDDH YCLDYWGQGT TLTVSSVEGG
401 SGGSGGSGGS GGVDIDIQLTQ SPAIMSASPG EKVTMTCRAS SSVSYMNWYQ
451 QKSGTSPKRW IYDTSKVASG VPYRFSGSGS GTSYSLTISS MEAEDAATYY
501 CQQWSSNPLT FGAGTKLELK HHHHHH*
```

Figure 3B (continued)

SEQ ID NO:29:

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT  
51 ACACTCCGCG CGCAGCTCG TGATGACCCA GACTCCACTC TCCCTGCCCTG  
101 TCAGTCTTGG AGATCAAGCC TCCATCTCTT GCAGATCTAG TCAGAGCCTT  
151 GTACACAGTA ATGGAACAC CTATTTACAT TGTACCTGC AGAAGCCAGG  
201 CCAGTCTCCA AAGTCTCTGA TCTACAAAGT TTCCAACCGA TTTTCTGGG  
251 TCCCAGACAG GTTCAGTGGC AGTGGATCAG GGACAGATTT CACACTCAAG  
301 ATCAGCAGAG TGGAGGCTGA GGATCTGGA GTTTATTTCT GCTCTCAAAG  
351 TACACATGTT CCGTACACGT TCGGAGGGG GACCAAGCTT GAGATCAAAG  
401 GTGGTGGTGG TTCTGGCGGC GCGGCTCCG GTGGTGGTGG TTCTGAGGTG  
451 CAGCTGCTCG AGCAGTCTGG AGCTGAGCTG GTAAGGCCCTG GGACTTCAGT  
501 GAAGCTGTCC TGCAAGGCTT CTGGCTACAC CTTCAACAAGC TATGGTTTAA  
551 GCTGGGTGAA GCAGAGAACT GGACAGGGCC TTGAGTGGAT TGGAGAGGTT  
601 TATCCTAGAA TTGGTAATGC TTAATAAAT GAGAAATTCA AGGCAAGGC  
651 CACACTGACT GCAGACAAAT CCTCCAGCAC AGCGTCCATG GAGCTCCGCA  
701 GCCTGACATC TGAGGACTCT GCGGTCTATT TCTGTGCAAG ACGGGGATCC  
751 TACGGTAGTA ACTACGACTG GTAATTGAT GTCTGGGGCC AAGGGACCAC  
801 GGTCAACCGTC TCCTCCGGAG GTGGTGGATC CGATATCAAA CTGCAGCAGT  
851 CAGGGGCTGA ACTGGCAAGA CCTGGGGCCT CAGTGAAGAT GTCCTGCAAG  
901 ACTTCTGGCT ACACCTTTAC TAGGTACACG ATGCACTGGG TAAAAACAGAG



Figure 3B (continued)

951 GCCTGGACAG GGTCTGGAAT GGATTGGATA CATTAATCCT AGCCGTGGTT  
1001 ATACTAATTA CAATCAGAAG TTCAAGGACA AGGCCACATT GACTACAGAC  
1051 AAATCCTCCA GCACAGCCTA CATGCAACTG AGCAGCCTGA CATCTGAGGA  
1101 CTCTGCAGTC TATTACTGTG CAAGATATTA TGATGATCAT TACTGCCCTTG  
1151 ACTACTGGG CCAAGGCACC ACTCTCACAG TCTCCTCAGT CGAAGGTGGA  
1201 AGTGGAGGTT CTGGTGGAAG TGGAGGTTCA GTGGAGTCG ACGACATTCA  
1251 GCTGACCCAG TCTCCAGCAA TCATGTCTGC ATCTCCAGG GAGAAGGTCA  
1301 CCATGACCTG CAGAGCCAGT TCAAGTGTA GTTACATGAA CTGTTACCAG  
1351 CAGAAGTCAG GCACCTCCCC CAAAAGATGG ATTTATGACA CATCCAAAGT  
1401 GGCTTCTGGA GTCCCTTATC GCTTCAGTGG CAGTGGGTCT GGGACCTCAT  
1451 ACTCTCTCAC AATCAGCAGC ATGGAGGCTG AAGATGCTGC CACTTATTAC  
1501 TGCCAACAGT GGAGTAGTAA CCGCTCACG TTCGGTGCTG GGACCAAGCT  
1551 GGAGCTGAAA CATCATCACC ATCACATTA G

## Figure 3C

3-1 (vLvH) x anti-CD3 (SEQ ID NO: 36)

```
1  MGWSCIIILFL VATAATGVHSE LVMTQSPSYL AASPGETITI NCRASKSISK
51  YLAWYQEKPG KTNKLLIYSG STLQSGIPSR FSGSGSGTDF TLTISSLEPE
101 DFAMYYCQQH NEYPYTFGGG TKLEIKGGG SGGGSGGGG SEVQLLEQSG
151 AELVKPGASV KISCKASGYA FTNYWLGWVK QRPCHGLEWI GDLFPGSGNT
201 HYNERFRGKA TLTADKSSST AFMQLSSLTS EDSAVYFCAR LRNWDEAMDY
251 WQGTTVTVS SGGGSDIKL QSGAELARP GASVKMSCKT SGYTFTRYTM
301 HWVKQRPQG LEWIGYINPS RGYTNYNQKF KDKATLTDDK SSSTAYMQLS
351 SLTSEDSAVY YCARYYDDHY CLDYWGQGT LTVSSVEGGS GSGGSGGSG
401 GVDDIQLTQS PAIMSASPG KVTMTCRASS SVSYMNWYQQ KSGTSPKRWI
451 YDTSKVASGV PYRFGSGSG TSYSLTISSM EAEDAATYYC QQWSSNPLTF
501 GAGTKLELKH HHHH*
```

Figure 3C (continued)

SEQ ID NO: 35

```
1  ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT
51  ACACCTCCGAG CTCGTATGA CCCAGTCTCC ATCTTATCTT GCTGCATCTC
101 CTGGAGAAAC CATTACTATT AATTGCAGGG CAAGTAAGAG CATTAGCAAA
151 TATTTAGCCT GGTATCAAGA GAAACCTGGG AAAACTAATA AGCTTCTTAT
201 CTACTCTGGA TCCACTTTGC AATCTGGAAT TCCATCAAGG TTCAGTGGCA
251 GTGGATCTGG TACAGATTTC ACTCTCACCA TCAGTAGCCT GGAGCCTGAA
301 GATTTTGCAA TGTATTACTG TCAACAGCAT AATGAATATC CGTACACGTT
351 CGAGGGGGG ACCAAGCTTG AGATCAAAGG TGGTGGTGGT TCTGGCGGCG
401 GCGGCTCCGG TGGTGGTGGT TCTGAGGTGC AGCTGCTCGA GCAGTCTGGA
451 GCTGAGCTGG TGAAACCTGG GGCTCAGTG AAGATATCCT GCAAGGCTTC
501 TGGATACGCC TTCACTAACT ACTGGCTAGG TTGGGTAAG CAGAGGCCCTG
551 GACATGGACT TGAGTGGATT GGAGATCTTT TCCCTGGAAG TGGTAATACT
601 CACTACAATG AGAGGTTTCA GGGCAAAGCC ACAC TGACTG CAGACAAATC
651 CTCGAGCACA GCCTTTATGC AGCTCAGTAG CCTGACATCT GAGGACTCTG
701 CTGTCTATTT CTGTGCAAGA TTGAGGAACT GGGACGAGGC TATGGACTAC
751 TGGGGCCAAG GGACCACGGT CACCGTCTCC TCCGGAGGTG GTGGATCCGA
801 TATCAAAC TG CAGCAGTCAG GGGCTGAACT GGCAAGACCT GGGGCCCTCAG
851 TGAAGATGTC CTGCAAGACT TCTGGCTACA CCTTTACTAG GTACACGATG
901 CACTGGGTAA AACAGAGGCC TGGACAGGGT CTGGAATGGA TTGGATACAT
```

**Figure 3C (continued)**

```
951 TAATCCTAGC CGTGGTTATA CTAATTACAA TCAGAAAGTTC AAGGACAAGG
1001 CCACATTGAC TACAGACAAA TCCTCCAGCA CAGCCTACAT GCAACTGAGC
1051 AGCCTGACAT CTGAGGACTC TGCAGTCTAT TACTGTGCAA GATATTATGA
1101 TGATCATTAC TGCCTTGACT ACTGGGGCCA AGGCACCACT CTCACAGTCT
1151 CCTCAGTCGA AGGTGGAAGT GGAGGTTCTG GTGGAAGTGG AGGTTCAGGT
1201 GGAGTCGACG ACATTCAGCT GACCCAGTCT CCAGCAATCA TGTCTGCATC
1251 TCCAGGGGAG AAGGTCACCA TGACCTGCAG AGCCAGTTCA AGTGTAAGTT
1301 ACATGAACTG GTACCAGCAG AAGTCAGGCA CCTCCCCCAA AAGATGGATT
1351 TATGACACAT CCAAAGTGGC TTCTGGAGTC CCTTATCGCT TCAGTGGCAG
1401 TGGGTCTGGG ACCTCATACT CTCACACAAT CAGCAGCATG GAGGCTGAAG
1451 ATGCTGCCAC TTATTACTGC CAACAGTGGA GTAGTAACCC GCTCACGTTT
1501 GGTGCTGGGA CCAAGCTGGA GCTGAAACAT CATCACCATC ATCATTAG
```

## Figure 3D

4-1 (vLvH) x anti-CD3 (SEQ ID NO: 39)

1	MGWSCIIILFL	VATATGVHSE	LVMTQSPSSL	SVSAGEKVTM	SCKSSQSLLN
51	SGNQKNYLAW	YQQKPGQPPK	LLIYGASTRE	SGVPDRFTGS	GSGETDFTLTI
101	SSVQAEDLAV	YYCQNDYSYP	YTFGGGTKLE	IKGGGGSGGG	GSGGGGSEVQ
151	LLEQSGAELV	RPGTSVKISC	KASGYAFTNY	WLGWVKQRPQ	HGLEWVGDI
201	PGSGNAHYNE	KFKGKATLTA	DKSSYTAYMQ	LSSLTSEDSA	VYFCARLRNW
251	DEAMDYWGQG	TTVTVSSGGG	GSDIKLQQSG	AELARPGASV	KMSCKTSGYT
301	FTRYTMHWVK	QRPQGGLWEI	GYINPSRGYT	NYNQKFKDKA	TLTTDKSSST
351	AYMQLSSLTS	EDSAVYYCAR	YYDDHYCLDY	WQGGTTLTVS	SVEGGSGGSG
401	GSGSGGVDD	IQLTQSPAIM	SASPGEKVTM	TCRASSSVSY	MNWIYQQKSGT
451	SPKRWIYDTS	KVASGVPPYRF	SGSGSGTSYS	LTISSMEAED	AATYYCQQWS
501	SNPLTFGAGT	KLELKHSHHHH	H*		

**Figure 3D (continued)****SEQ ID NO: 38:**

```
1  ATGGGATGGA  GCTGTATCAT  CCTCTTCTTG  GTAGCAACAG  CTACAGGTGT
51  ACACCTCCGAG  CTCGTGATGA  CACAGTCTCC  ATCCTCCCCTG  AGTGTGTCAG
101 CAGGAGAGAA  GGTCACTATG  AGCTGCAAGT  CCAGTCAGAG  TCTGTTAAAC
151 AGTGGAATC  AAAAGAACTA  CTTGGCCTGG  TACCAGCAGA  AACCAGGGCA
201 GCCTCCTAAA  CTGTTGATCT  ACGGGGCATC  CACTAGGGAA  TCTGGGGTCC
251 CTGATCGCTT  CACAGGCAGT  GGATCTGGAA  CAGATTTCAC  TCTCACCATC
301 AGCAGTGTGC  AGGCTGAAGA  CCTGGCAGTT  TATTACTGTC  AGAATGATTA
351 TAGTTATCCG  TACACGTTCC  GAGGGGGAC  CAAGCTTGAG  ATCAAAGGTG
401 GTGGTGGTTC  TGGCGCGGC  GGCTCCGGTG  GTGGTGGTTC  TGAGGTGCAG
451 CTGCTCGAGC  AGTCTGGAGC  TGAGCTGGTA  AGGCCTGGGA  CTTCAGTGAA
501 GATATCCTGC  AAGGCTTCTG  GATACGCCCT  CACTAACTAC  TGGCTAGGTT
551 GGGTTAAGCA  GAGGCCCTGGA  CATGGACTTG  AATGGGTGG  AGATATTTTC
601 CCTGGAAGTG  GTAA TGCTCA  CTACAATGAG  AAGTCAAGG  GCAAAGCCAC
651 ACTGACTGCA  GACAAGTCCT  CGTACACAGC  CTATATGCAG  CTCAGTAGCC
701 TGACATCTGA  GGACTCTGCT  GTCTATTCT  GTGCAAGATT  GCGGAAC TGG
751 GACGAGGCTA  TGGACTACTG  GGCCCAAGGG  ACCACGGTCA  CCGTCTCCTC
801 CGGAGGTGGT  GGATCCGATA  TCAA AACTGCA  GCAGTCAGGG  GCTGAAC TGG
851 CAAGACCTGG  GGCTCAGTG  AAGATGTCCT  GCAAGACTTC  TGGCTACACC
901 TTTACTAGGT  ACACGATGCA  CTGGGTAAAA  CAGAGGCCCTG  GACAGGGTCT
```

Figure 3D (continued)

```
951 GGAATGGATT GGATACATTA ATCCTAGCCG TGGTTATACT AATTACAATC
1001 AGAAGTTCAA GGACAAGGCC ACATTGACTA CAGACAAATC CTCACGCACA
1051 GCCTACATGC AACTGAGCAG CCTGACATCT GAGGACTCTG CAGTCTATTA
1101 CTGTGCAAGA TATTATGATG ATCATTACTG CCTTGACTAC TGGGGCCAAG
1151 GCACCACTCT CACAGTCTCC TCAGTCGAAG GTGGAAGTGG AGGTTCTGGT
1201 GGAAGTGGAG GTTCAGGTGG AGTCGACGAC ATTCAAGCTGA CCCAGTCTCC
1251 AGCAATCATG TCTGCATCTC CAGGGGAGAA GGTCAACCATG ACCTGCAGAG
1301 CCAGTTCAAG TGTAAGTTAC ATGAACTGGT ACCAGCAGAA GTCAGGCACC
1351 TCCCCCAAAA GATGGATTTA TGACACATCC AAAGTGGCTT CTGGAGTCCC
1401 TTATCGCTTC AGTGGCAGTG GGTCTGGGAC CTCATACTCT CTCACAATCA
1451 GCAGCATGGA GGCTGAAGAT GCTGCCACTT ATTACTGCCA ACAGTGGAGT
1501 AGTAACCCGC TCACGTTCCG TGCTGGGACC AAGCTGGAGC TGAAACATCA
1551 TCACCATCAT CATTAG
```

## Figure 3E

5-10 (vLvH) x anti-CD3 (SEQ ID NO: 44)

```
1  MGWSCIIILFL VATAATGVHSE LVMTQSPSSL TVTAGEKVTM SCKSSQSLLN
51  SGNQKNYLTW YQKPGQPPK LLIYWASTRE SGVPDRFTGS GSGTDFTLTI
101 SSVQAEDLAV YYCQNDYSYP LTFGAGTKLE IKGGGGSGGG GSGGGGSEVQ
151 LLEQSGAELV RPGTSVKISC KASGYAFTNY WLGVVKORPG HGLEWIGDIF
201 PGSGNIHYNE KFKGKATLTA DKSSSTAYMQ LSSLTFEDSA VYFCARLRNW
251 DEPMDYWGQG TTVTVSSGGG GSDIKLQSG AELARPGASV KMCKTSGYT
301 FTRYTMHWVK QRPQGLEWI GYINPSRGYT NYNQKFKDKA TLTTDKSSST
351 AYMQLSSLTS EDSAVYYCAR YYDDHYCLDY WGQGTTLTVS SVEGGSGSG
401 GSGSGGVDD IQLTQSPAIM SASPGEKVTM TCRASSSVSY MNWYQQKSGT
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## Figure 3E (continued)

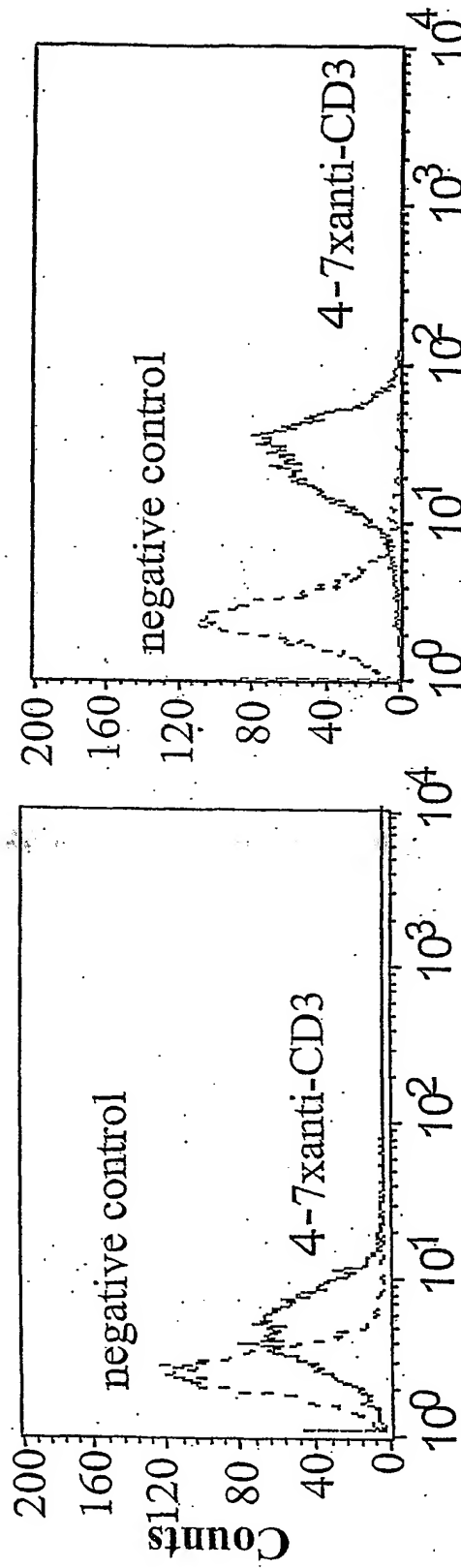
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101 CAGGAGAGAA  GGTCACTATG  AGCTGCAAGT  CCAGTCAGAG  TCTGTTAAAC
151 AGTGGAATC  AAAAGAACTA  CTTGACCCTG  TACCAGCAGA  AACCAAGGCA
201 GCCTCCTAAA  CTGTTGATCT  ACTGGGCATC  CACTAGGGAA  TCTGGGGTCC
251 CTGATCGCTT  CACAGGCAGT  GGATCTGGAA  CAGATTTCAC  TCTCACCATC
301 AGCAGTGTGC  AGGCTGAAGA  CCTGGCAGTT  TATTACTGTC  AGAATGATTA
351 TAGTTATCCG  CTCACGTTTC  GTGCTGGGAC  CAAGCTTGAG  ATCAAAGGTG
401 GTGGTGGTTC  TGGCGCGGCG  GGCTCCGGTG  GTGGTGGTTC  TGAGGTGCAG
451 CTGCTCGAGC  AGTCTGGAGC  TGAGCTGGTA  AGGCCTGGGA  CTTCAGTGAA
501 GATATCCTGC  AAGGCTTCTG  GATACGCCTT  CACTAACTAC  TGGCTAGGTT
551 GGGTAAAGCA  GAGGCCCTGGA  CATGGACTTG  AGTGGATTGG  AGATATTTC
601 CCTGGAAGTG  GTAATATCCA  CTACAATGAG  AAGTCAAGG  GCAAAGCCAC
651 ACTGACTGCA  GACAAATCTT  CGAGCACAGC  CTATATGCAG  CTCAGTAGCC
701 TGACATTTGA  GGACTCTGCT  GTCTATTTC  GTGCAAGACT  GAGGAACTGG
751 GACGAGCCTA  TGGACTACTG  GGGCCAAGGG  ACCACGGTCA  CCGTCTCCTC
801 CGGAGGTGGT  GGATCCGATA  TCAAACTGCA  GCAGTCAGGG  GCTGAACTGG
851 CAAGACCTGG  GGCCTCAGTG  AAGATGTCCT  GCAAGACTTC  TGGCTACACC
901 TTTACTAGGT  ACACGATGCA  CTGGGTAAAA  CAGAGGCCCTG  GACAGGGTCT
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Figure 3E (continued)

```
951 GGAATGGATT GGATACATTA ATCCTAGCCG TGGTTATACT AATTACAATC
1001 AGAAGTTCAA GGACAAGGCC ACATTGACTA CAGACAAATC CTCCAGCACA
1051 GCCTACATGC AACTGAGCAG CCTGACATCT GAGGACTCTG CAGTCTATTA
1101 CTGTGCAAGA TATTATGATG ATCATTACTG CCTTGACTAC TGGGGCCAAG
1151 GCACCACTCT CACAGTCTCC TCAGTCGAAG GTGGAAGTGG AGGTTCTGGT
1201 GGAAGTGGAG GTTCAGGTGG AGTCGACGAC ATTCAGCTGA CCCAGTCTCC
1251 AGCAATCATG TCTGCATCTC CAGGGAGAA GGTCACCATG ACCTGCAGAG
1301 CCAGTTC AAG TGTAAGTTAC ATGAACTGGT ACCAGCAGAA GTCAGGCACC
1351 TCCCCCAAAA GATGGATTTA TGACACATCC AAAGTGGCTT CTGGAGTCCC
1401 TTATCGCTTC AGTGGCAGTG GGTCTGGGAC CTCATACTCT CTCACAATCA
1451 GCAGCATGGA GGCTGAAGAT GCTGCCACTT ATTACTGCCA ACAGTGGAGT
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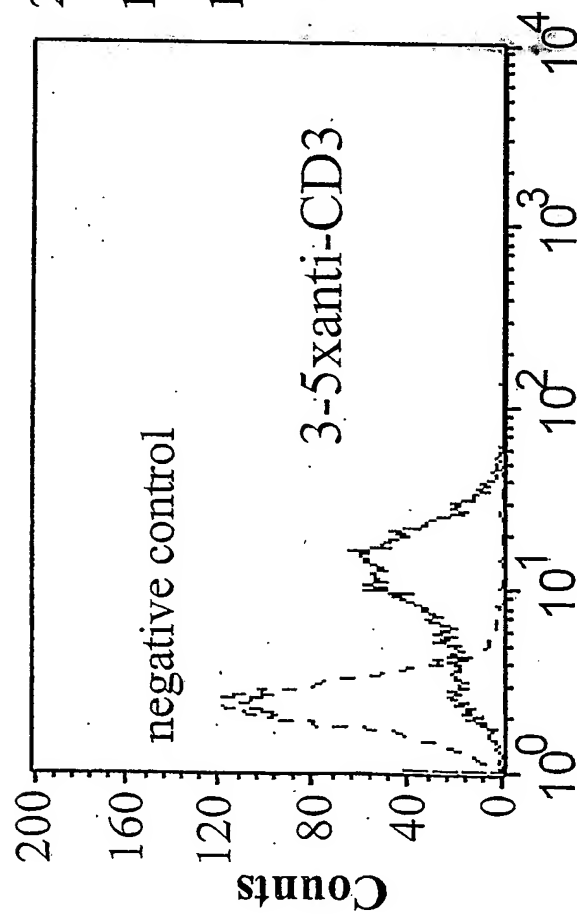
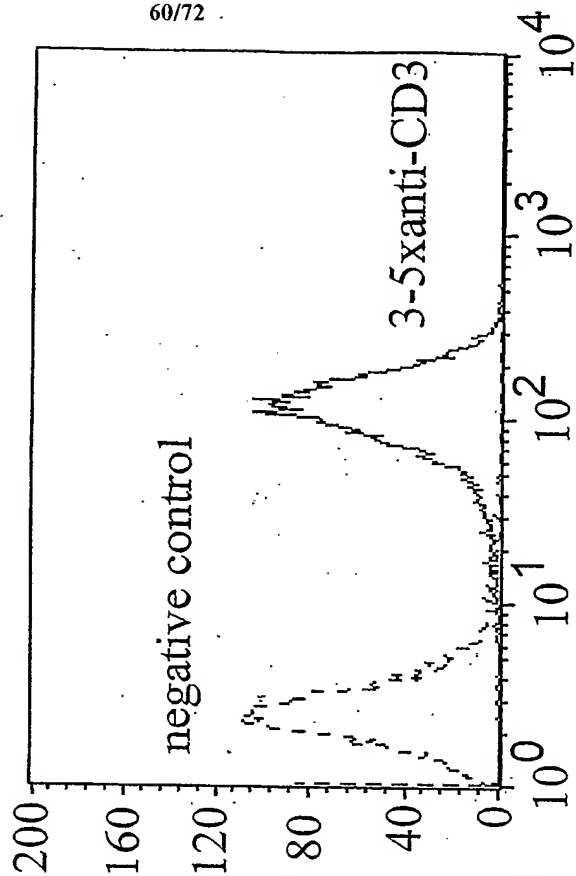
Figure 4A

CD3 Binding (Jurkat cells)EpCAM Binding (Kato cells)

4-7xanti-CD3

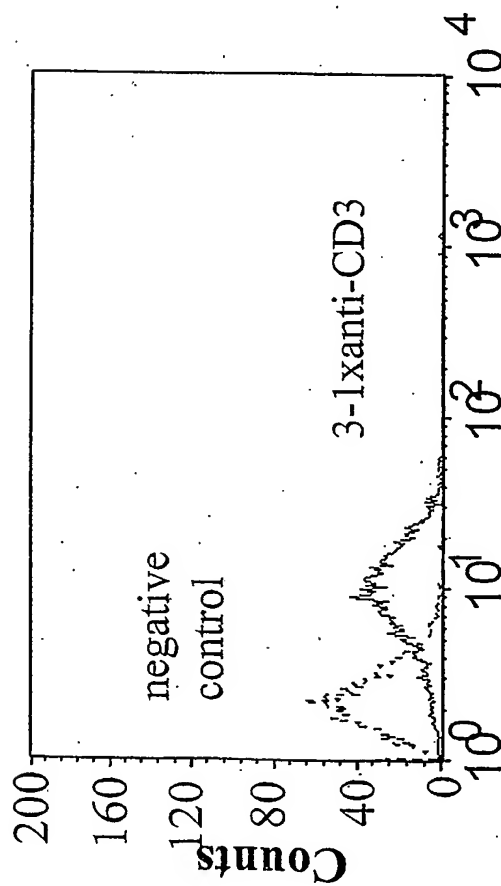
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60/72

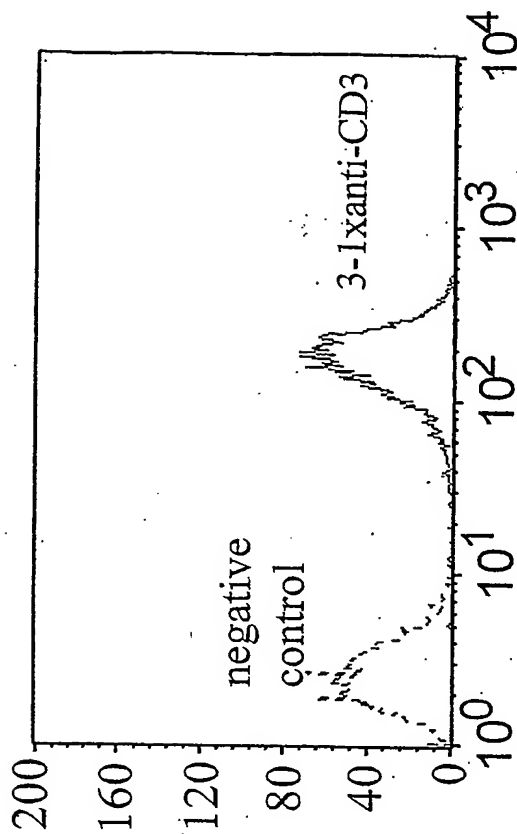
**Figure 4B****CD3 Binding (Jurkat cells)****EpCAM Binding (Kato cells)****3-5xanti-CD3****(SEQ ID NO: 30)**

**Figure 4C**

**CD3 Binding (Jurkat cells)**



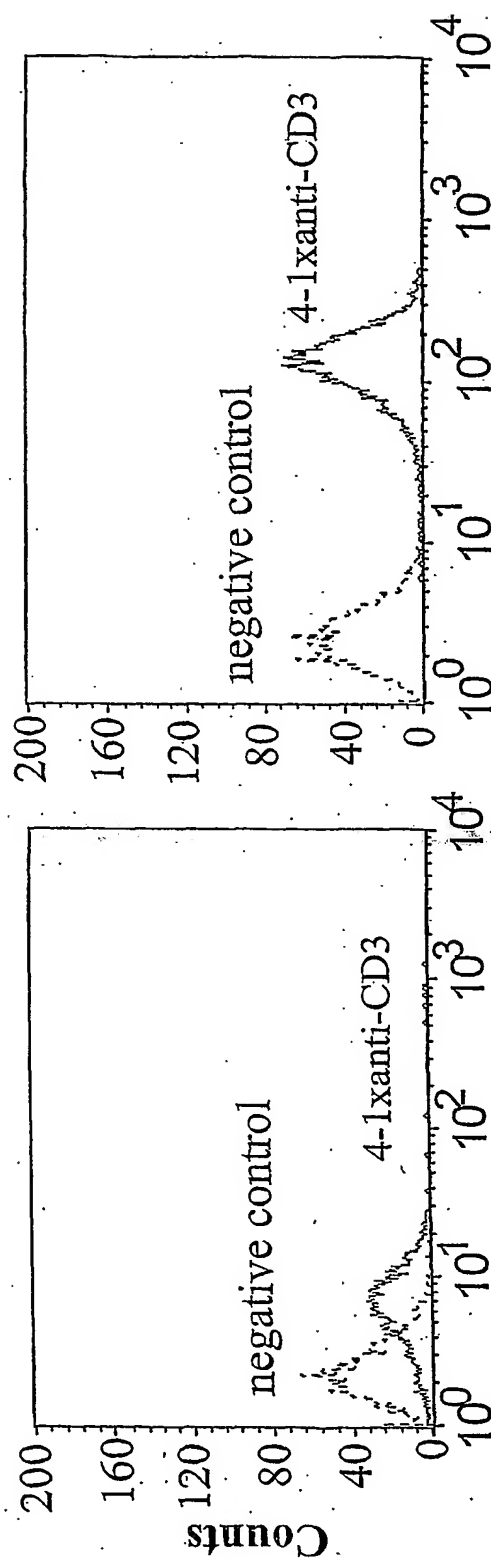
**EpCAM Binding (Kato cells)**



**3-1xanti-CD3**

**(SEQ ID NO:36)**

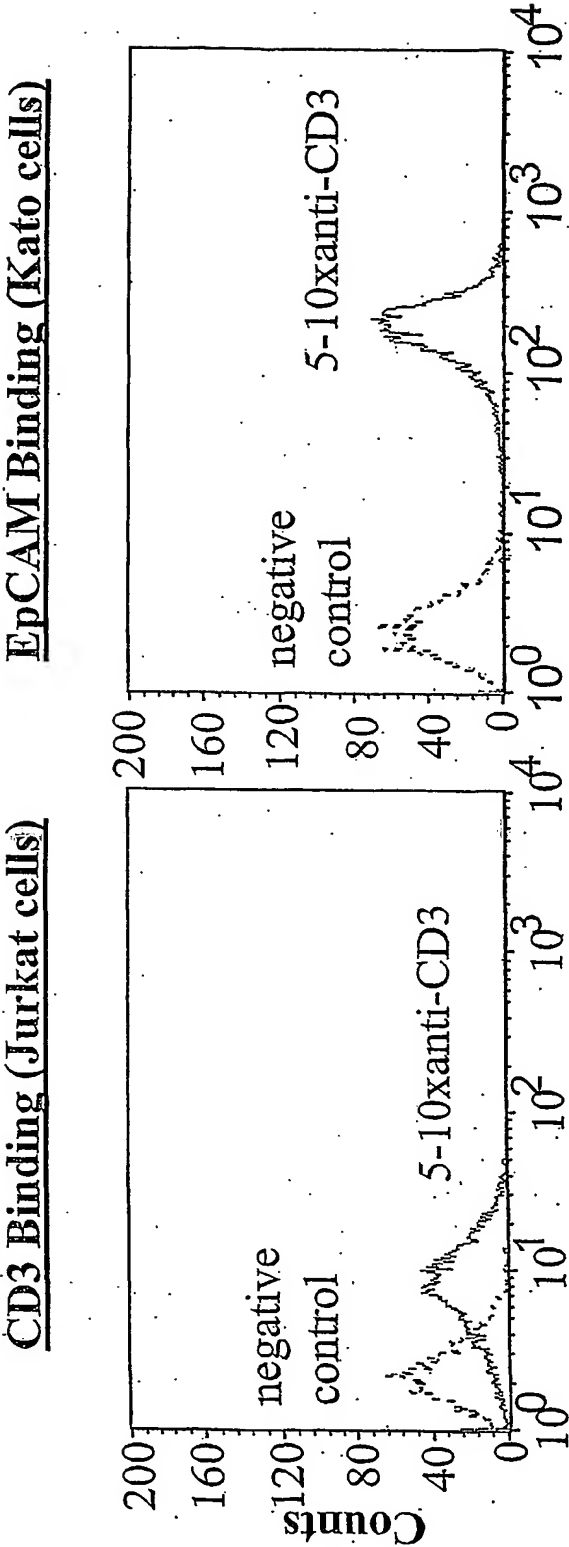
Figure 4D

CD3 Binding (Jurkat cells)EpCAM Binding (Kato cells)

4-1xanti-CD3

(SEQ ID NO: 39)

Figure 4E



5-10xanti-CD3  
(SEQ ID NO: 44)

Figure 5

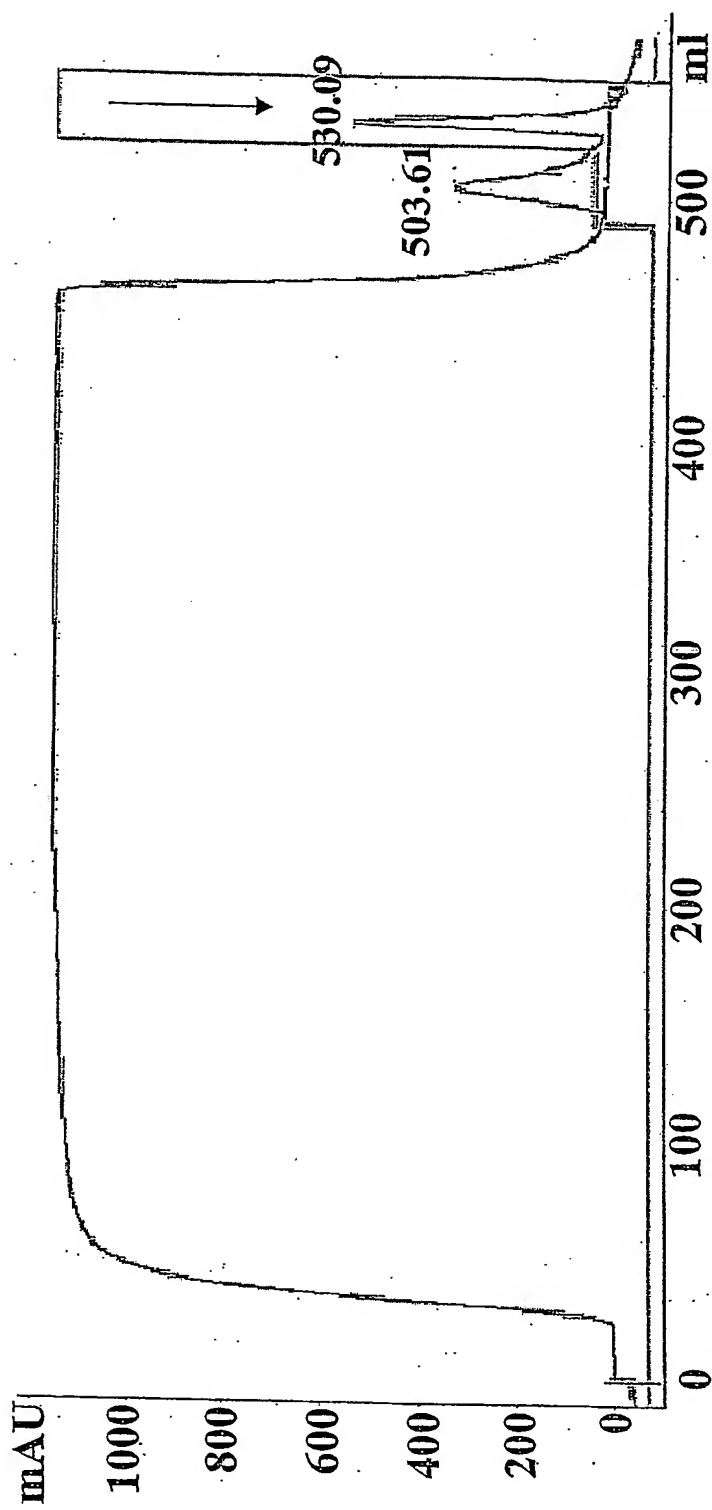
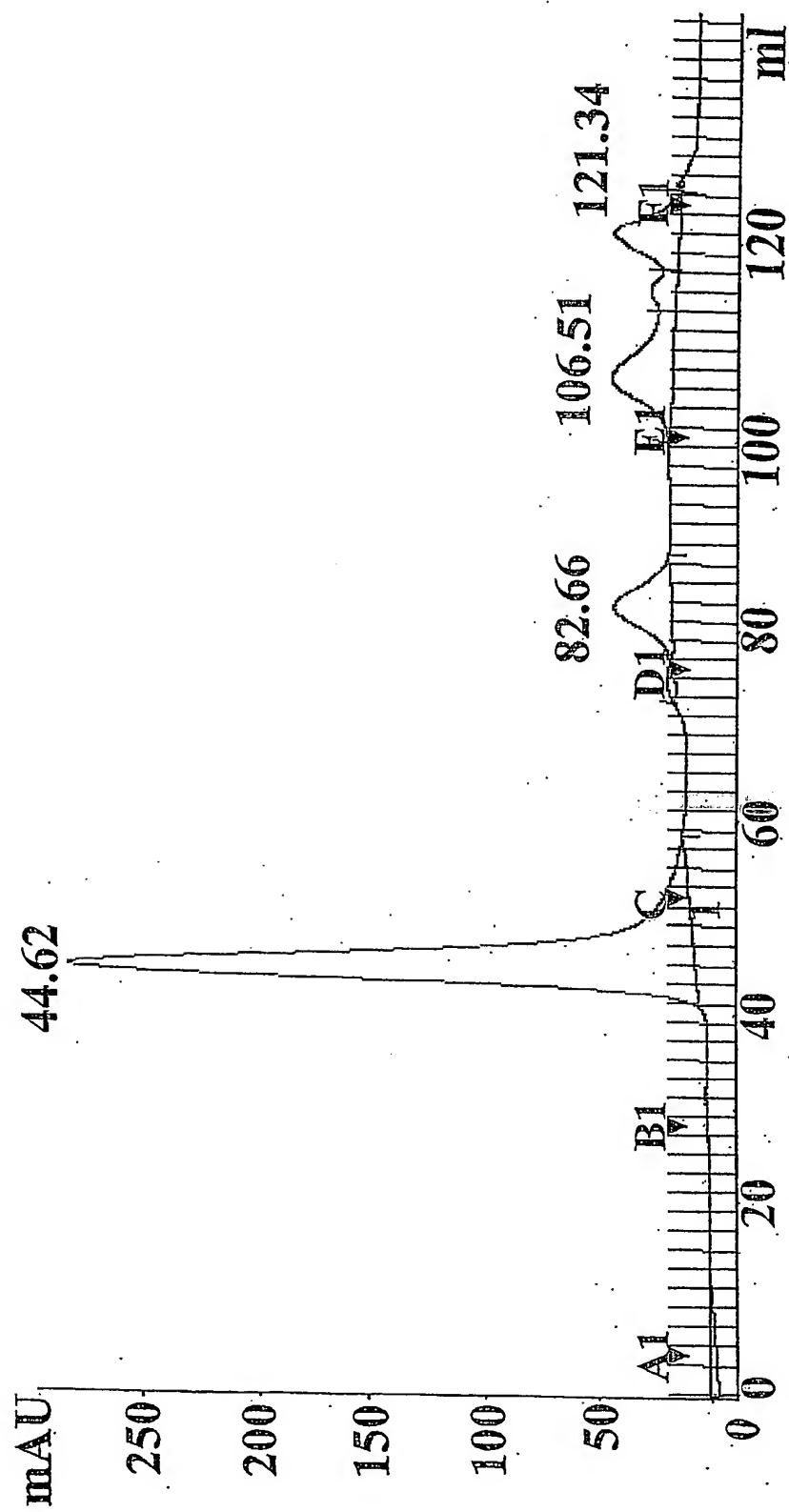




Figure 6



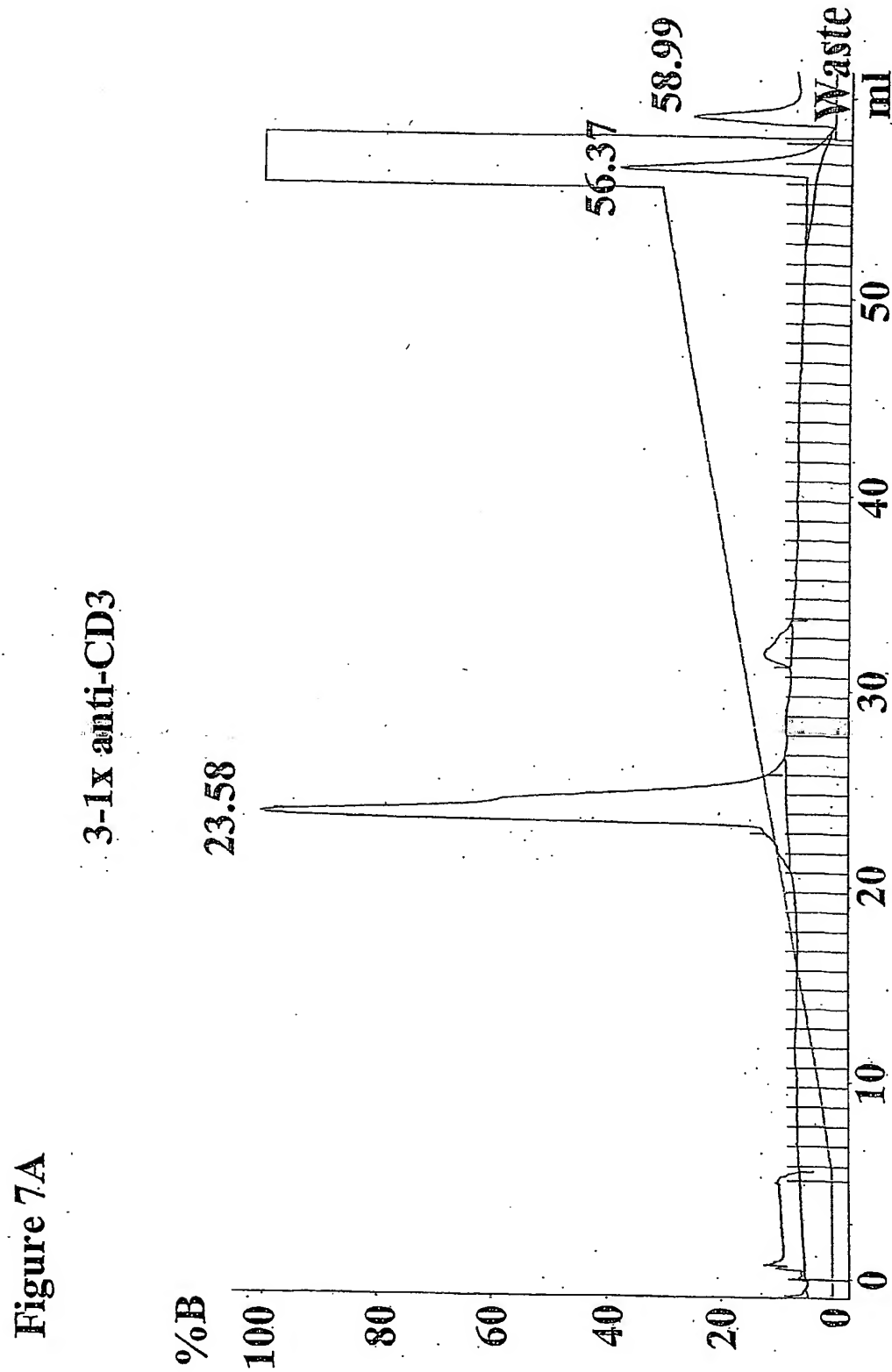


Figure 7B

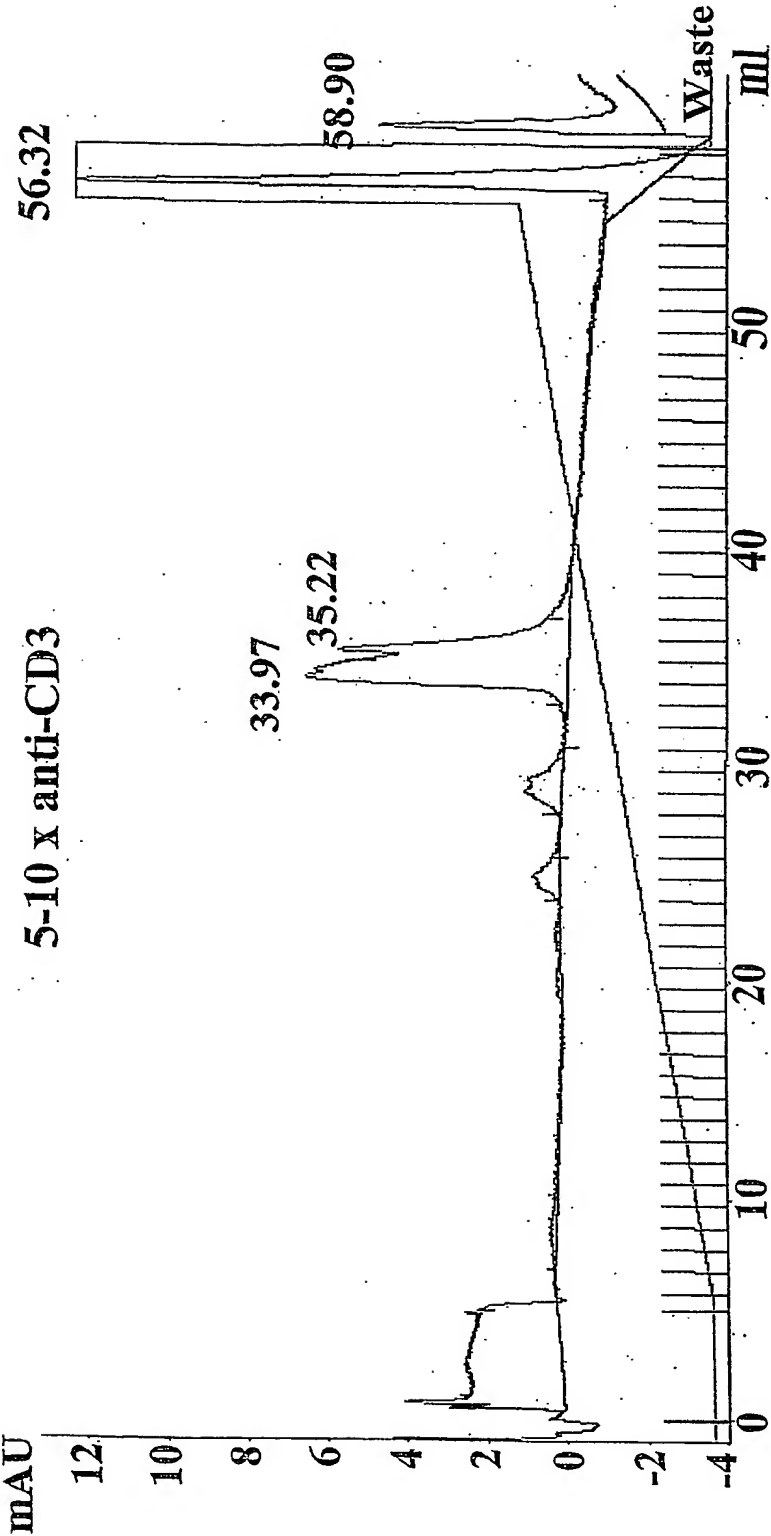
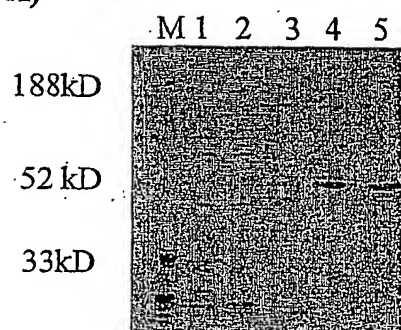
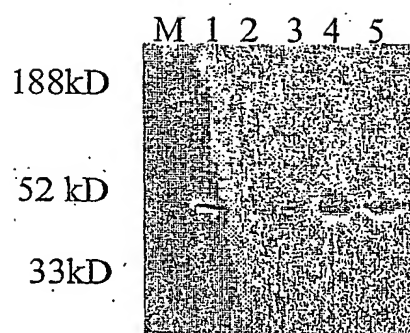


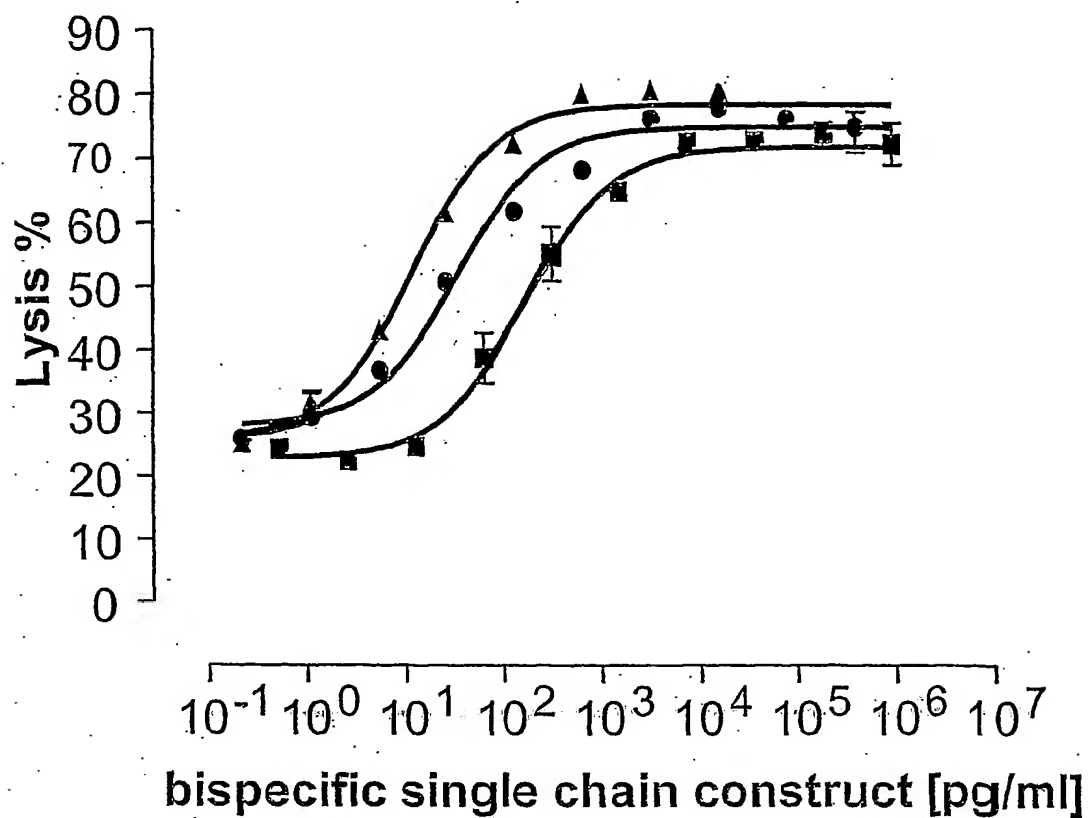
Figure 8

A)

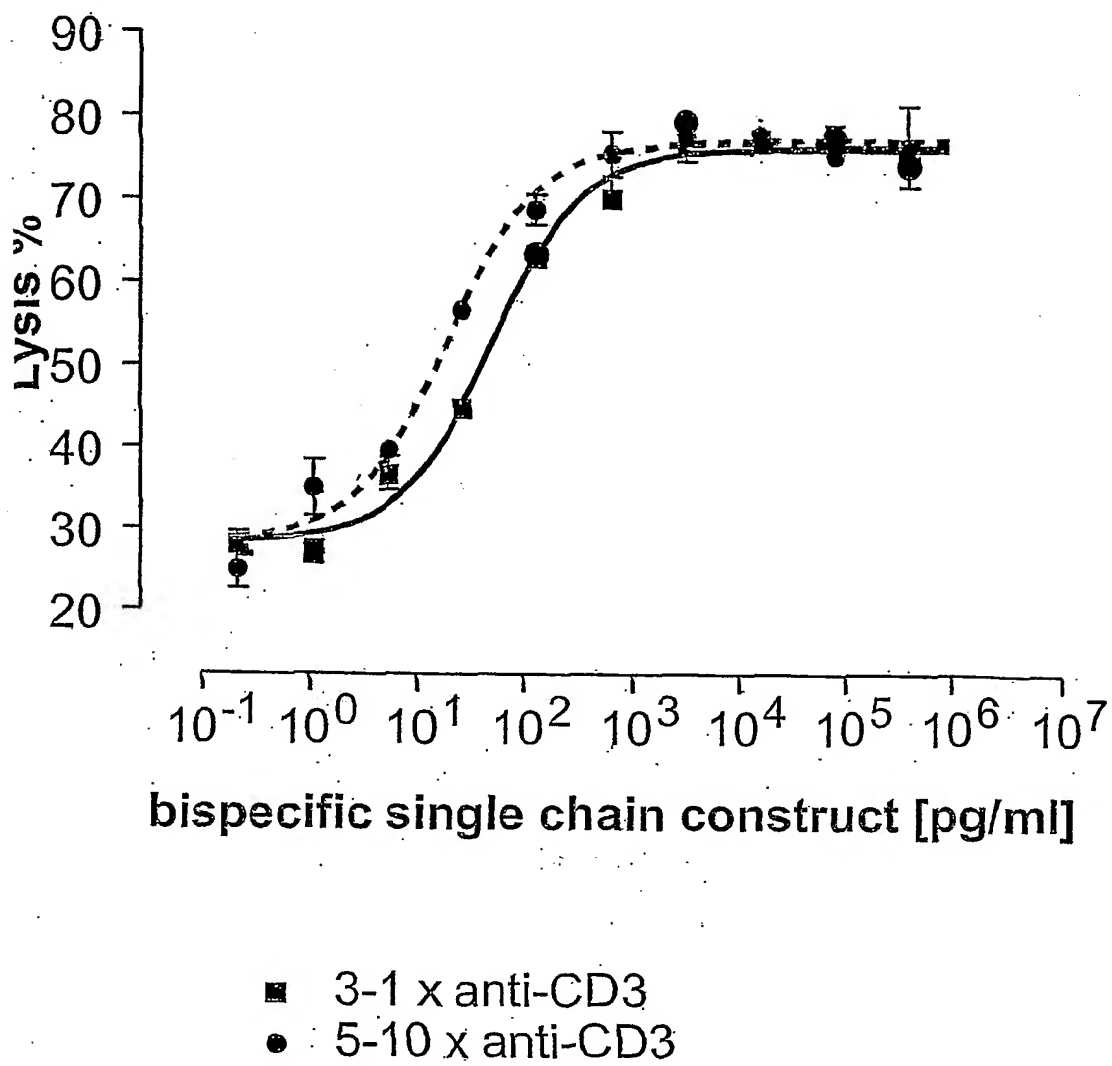


B)



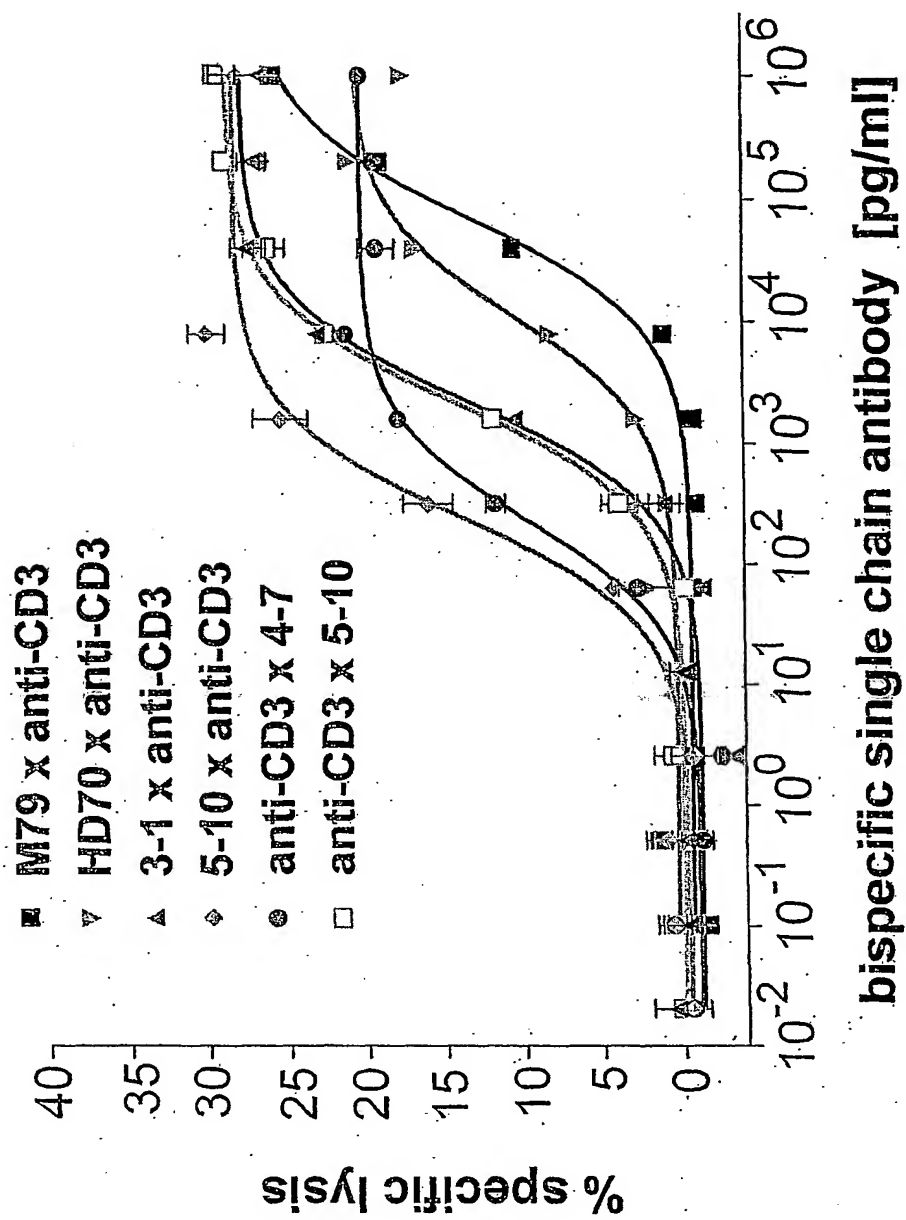
**Figure 9**

- anti-CD3x3-1
- anti-CD3 x 5-10
- ▲ anti-CD3 x 4-7

**Figure 10**

**Figure 11A**

3-1	LR <b>NW</b> DEAMDY
4-1	LR <b>NW</b> DEAMDY
5-10	LR <b>NW</b> DEPMDY
3-5	RGSYGS <b>NYD</b> WYFDV
4-7	RGSYDT <b>NYD</b> WYFDV
M79	MENWSFAY
HD70	DMGWGSGWRPYYYYGMDV
3B10	FTSPDY

**Figure 11B**



SEQUENCE LISTING<sup>1</sup>

<110> Micromet AG

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<130> H1656 PCT

<150> EP 03012133.9

<151> 2003-05-31

<150> EP 03012134.7

<151> 2003-05-31

<160> 101

<170> PatentIn version 3.1

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<213> artificial sequence

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2

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&lt;211&gt; 506

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

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&lt;223&gt; CD3 VHVL aL x 4-7 VHVL

&lt;400&gt; 2

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Ser Val Lys Met Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Arg Tyr  
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Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile  
35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe  
50 55 60

Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr  
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Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys  
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly  
100 105 110

Thr Thr Leu Thr Val Ser Ser Val Glu Gly Gly Ser Gly Gly Ser Gly  
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Gly Ser Gly Gly Ser Gly Gly Val Asp Asp Ile Gln Leu Thr Gln Ser  
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Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys  
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Arg Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser  
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Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser  
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Gly Val Pro Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser  
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Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys  
210 215 220

Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu  
225 230 235 240

Glu Leu Lys Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln  
245 250 255

Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala Ser Val Lys Leu Ser Cys  
260 265 270

Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr Gly Leu Ser Trp Val Lys  
275 280 285

Gln Arg Pro Gly Gln Val Leu Glu Trp Ile Gly Glu Val Tyr Pro Arg  
290 295 300

Ile Gly Asn Ala Tyr Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu  
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325 330 335

Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg Arg Gly Ser Tyr  
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4

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Val Thr Val Ser Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly  
 370 375 380

Gly Gly Gly Ser Glu Leu Val Met Thr Gln Thr Pro Leu Ser Leu Pro  
 385 390 395 400

Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser  
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Leu Val His Ser Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys  
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Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe  
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Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe  
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Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe  
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5

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&lt;212&gt; PRT

&lt;213&gt; artificial sequence

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&lt;223&gt; CD3 VHVL aL x 5-10 VHVL

&lt;400&gt; 4

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Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
1       5       10      15

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Ser Val Lys Met Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Arg Tyr
20      25      30

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Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35      40      45

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Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe

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Ala	Arg	Tyr	Tyr 100	Asp	Asp	His	Tyr	Cys 105	Leu	Asp	Tyr	Trp	Gly 110	Gln	Gly
Thr	Thr	Leu 115	Thr	Val	Ser	Ser	Val 120	Glu	Gly	Gly	Ser	Gly 125	Gly	Ser	Gly
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7

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Gly Asn Gln Lys Asn Tyr Leu Thr Trp Tyr Gln Gln Lys Pro Gly Gln  
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<400> 6

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gtcagtcctg gagatcaagc ctccatctct tgcagatcta gtcagagcct tgtacacagt	1260



aatggaaca cctatttaca ttggtacctg cagaagccag gccagtctcc aaagctcctg 1320  
 atctaāaaag tttccaaccg attttctggg gtcccagaca ggttcagtgg cagtggatca 1380  
 gggacagatt tcacactcaa gatcagcaga gtggaggctg aggatctggg agtttatttc 1440  
 tgctctcaaa gtacacatgt tccgtacacg ttcggagggg ggaccaagct tgagatcaaa 1500  
 catcatcacc atcatcatta g 1521

<210> 8

<211> 506

<212> PRT

<213> artificial sequence

<220>

<223> CD3 VHVL aL Ser x 4-7 VHVL

<400> 8

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala  
 1 5 10 15

Ser Val Lys Met Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Arg Tyr  
 20 25 30

Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile  
 35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe  
 50 55 60

Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr  
 65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys  
 85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Ser Leu Asp Tyr Trp Gly Gln Gly  
 100 105 110

Thr Thr Leu Thr Val Ser Ser Val Glu Gly Gly Ser Gly Gly Ser Gly  
 115 120 125

Gly Ser Gly Gly Ser Gly Gly Val Asp Asp Ile Gln Leu Thr Gln Ser  
 130 135 140

Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys  
 145 150 155 160

Arg Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser

165										170 <sup>10</sup>					175				
Gly	Thr	Ser	Pro 180	Lys	Arg	Trp	Ile	Tyr 185	Asp	Thr	Ser	Lys	Val 190	Ala	Ser				
Gly	Val	Pro 195	Tyr	Arg	Phe	Ser	Gly 200	Ser	Gly	Ser	Gly	Thr 205	Ser	Tyr	Ser				
Leu	Thr 210	Ile	Ser	Ser	Met	Glu 215	Ala	Glu	Asp	Ala	Ala 220	Thr	Tyr	Tyr	Cys				
Gln 225	Gln	Trp	Ser	Ser	Asn 230	Pro	Leu	Thr	Phe	Gly 235	Ala	Gly	Thr	Lys	Leu 240				
Glu	Leu	Lys	Ser	Gly 245	Gly	Gly	Gly	Ser	Glu 250	Val	Gln	Leu	Leu	Glu 255	Gln				
Ser	Gly	Ala	Glu 260	Leu	Ala	Arg	Pro	Gly 265	Ala	Ser	Val	Lys	Leu 270	Ser	Cys				
Lys	Ala	Ser 275	Gly	Tyr	Thr	Phe	Thr 280	Asn	Tyr	Gly	Leu	Ser 285	Trp	Val	Lys				
Gln	Arg 290	Pro	Gly	Gln	Val	Leu 295	Glu	Trp	Ile	Gly	Glu 300	Val	Tyr	Pro	Arg				
Ile 305	Gly	Asn	Ala	Tyr	Tyr 310	Asn	Glu	Lys	Phe	Lys 315	Gly	Lys	Ala	Thr	Leu 320				
Thr	Ala	Asp	Lys	Ser 325	Ser	Ser	Thr	Ala	Ser 330	Met	Glu	Leu	Arg	Ser 335	Leu				
Thr	Ser	Glu	Asp 340	Ser	Ala	Val	Tyr	Phe 345	Cys	Ala	Arg	Arg	Gly 350	Ser	Tyr				
Asp	Thr	Asn 355	Tyr	Asp	Trp	Tyr	Phe 360	Asp	Val	Trp	Gly	Gln 365	Gly	Thr	Thr				
Val	Thr 370	Val	Ser	Ser	Gly	Gly 375	Gly	Gly	Ser	Gly	Gly 380	Gly	Gly	Ser	Gly				
Gly 385	Gly	Gly	Ser	Glu	Leu 390	Val	Met	Thr	Gln	Thr 395	Pro	Leu	Ser	Leu	Pro 400				
Val	Ser	Leu	Gly	Asp 405	Gln	Ala	Ser	Ile	Ser 410	Cys	Arg	Ser	Ser	Gln 415	Ser				
Leu	Val	His	Ser 420	Asn	Gly	Asn	Thr	Tyr 425	Leu	His	Trp	Tyr	Leu 430	Gln	Lys				
Pro	Gly	Gln 435	Ser	Pro	Lys	Leu	Leu 440	Ile	Tyr	Lys	Val	Ser 445	Asn	Arg	Phe				

Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe  
 450 455 460

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe  
 465 470 475 480

Cys Ser Gln Ser Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys  
 485 490 495

Leu Glu Ile Lys His His His His His His  
 500 505

<210> 9

<211> 1512

<212> DNA

<213> artificial sequence

<220>

<223> CD3 VHVL aL Ser x 5-10 VHVL

<400> 9

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tcctgcaaga cttctggcta cacctttact aggtacacga tgcactgggt aaaacagagg	120
cctggacagg gtctggaatg gattggatac attaatccta gccgtgggta tactaattac	180
aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac	240
atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat	300
gatgatcatt actcccttga ctactggggc caaggcacca ctctcacagt ctctcagtc	360
gaagggtggaa gtggaggttc tgggtggaagt ggaggttcag gtggagtcga cgacattcag	420
ctgacccagt ctccagcaat catgtctgca tctccagggg agaaggtcac catgacctgc	480
agagccagtt caagtgtgaa ttacatgaac tgggtaccagc agaagtcagg cacctcccc	540
aaaagatgga tttatgacac atccaaagtg gcttctggag tcccttatcg cttcagtggc	600
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acttattact gccaacagtg gagtagtaac ccgctcacgt tcggtgctgg gaccaagctg	720
gagctgaaat ccggaggtgg tggatccgag gtgcagctgc tcgagcagtc tggagctgag	780
ctggtaaggc ctgggacttc agtgaagata tcctgcaagg cttctggata cgccttact	840
aactactggc taggttgggt aaagcagagg cctggacatg gacttgagtg gattggagat	900
atcttccctg gaagtggtaa tatccactac aatgagaagt tcaagggcaa agccacactg	960
actgcagaca aatcttcgag cacagcctat atgcagctca gtagcctgac atttgaggac	1020
tctgctgtct atttctgtgc aagactgagg aactgggacg agcctatgga ctactggggc	1080

12

caagggacca cggtcaccgt ctcctcaggt ggtggtggtt ctggcggcgg cggctccggt	1140
ggtggtggtt ctgagctcgt gatgacacag tctccatcct ccctgactgt gacagcagga	1200
gagaagggtca ctatgagctg caagtccagt cagagtctgt taaacagtgg aaatcaaaag	1260
aactacttga cctggtacca gcagaaacca gggcagcctc ctaaactgtt gatctactgg	1320
gcattccacta gggaaatctgg ggtccctgat cgcttcacag gcagtggatc tggaacagat	1380
ttcactctca ccatacagcag tgtgcaggct gaagacctgg cagtttatta ctgtcagaat	1440
gattatagtt atccgctcac gttcgggtgct gggaccaagc ttgagatcaa acatcatcac	1500
catcatcatt ag	1512

&lt;210&gt; 10

&lt;211&gt; 503

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; CD3 VHVL aL Ser x 5-10 VHVL

&lt;400&gt; 10

Asp	Ile	Lys	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Leu	Ala	Arg	Pro	Gly	Ala
1				5					10					15	

Ser	Val	Lys	Met	Ser	Cys	Lys	Thr	Ser	Gly	Tyr	Thr	Phe	Thr	Arg	Tyr
			20					25					30		

Thr	Met	His	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile
		35					40					45			

Gly	Tyr	Ile	Asn	Pro	Ser	Arg	Gly	Tyr	Thr	Asn	Tyr	Asn	Gln	Lys	Phe
	50					55					60				

Lys	Asp	Lys	Ala	Thr	Leu	Thr	Thr	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr
65					70					75				80	

Met	Gln	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Tyr	Cys
			85					90						95	

Ala	Arg	Tyr	Tyr	Asp	Asp	His	Tyr	Ser	Leu	Asp	Tyr	Trp	Gly	Gln	Gly
			100					105					110		

Thr	Thr	Leu	Thr	Val	Ser	Ser	Val	Glu	Gly	Gly	Ser	Gly	Gly	Ser	Gly
		115					120					125			

Gly	Ser	Gly	Gly	Ser	Gly	Gly	Val	Asp	Asp	Ile	Gln	Leu	Thr	Gln	Ser
	130					135					140				

Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys  
 145 150 155 160

Arg Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser  
 165 170 175

Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser  
 180 185 190

Gly Val Pro Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser  
 195 200 205

Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys  
 210 215 220

Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu  
 225 230 235 240

Glu Leu Lys Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln  
 245 250 255

Ser Gly Ala Glu Leu Val Arg Pro Gly Thr Ser Val Lys Ile Ser Cys  
 260 265 270

Lys Ala Ser Gly Tyr Ala Phe Thr Asn Tyr Trp Leu Gly Trp Val Lys  
 275 280 285

Gln Arg Pro Gly His Gly Leu Glu Trp Ile Gly Asp Ile Phe Pro Gly  
 290 295 300

Ser Gly Asn Ile His Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu  
 305 310 315 320

Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu  
 325 330 335

Thr Phe Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg Leu Arg Asn Trp  
 340 345 350

Asp Glu Pro Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser  
 355 360 365

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
 370 375 380

Glu Leu Val Met Thr Gln Ser Pro Ser Ser Leu Thr Val Thr Ala Gly  
 385 390 395 400

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser  
 405 410 415

Gly Asn Gln Lys Asn Tyr Leu Thr Trp Tyr Gln Gln Lys Pro Gly Gln

14

420                      425                      430

Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val  
           435                      440                      445

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr  
           450                      455                      460

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn  
 465                      470                      475                      480

Asp Tyr Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Ile  
           485                      490                      495

Lys His His His His His  
           500

&lt;210&gt; 11

&lt;211&gt; 1485

&lt;212&gt; DNA

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; CD3 VHVL stL x 3-1 VHVL

&lt;400&gt; 11

gatatcaaac tgcagcagtc aggggctgaa ctggcaagac ctggggcctc agtgaagatg	60
tcctgcaaga cttctggcta cacctttact aggtacacga tgcactgggt aaaacagagg	120
cctggacagg gtctggaatg gattggatac attaatccta gccgtgggta tactaattac	180
aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac	240
atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat	300
gatgatcatt actgccttga ctactggggc caaggcacca ctctcacagt ctctcagggt	360
ggtggtggtt ctggcggcgg cggctccggg ggtggtggtt ctgacattca gctgaccag	420
tctccagcaa tcatgtctgc atctccaggg gagaagggtca ccatgacctg cagagccagt	480
tcaagtgtaa gttacatgaa ctggtaccag cagaagtcag gcacctcccc caaaagatgg	540
atttatgaca catccaaagt ggcttctgga gtcccttata gcttcagtgg cagtgggtct	600
gggacctcat actctctcac aatcagcagc atggaggctg aagatgctgc cacttattac	660
tgccaacagt ggagtagtaa cccgctcacg ttcgggtgctg ggaccaagct ggagctgaaa	720
tccggagggtg gtggatccga ggtgcagctg ctcgagcagt ctggagctga gctggtgaaa	780
cctggggcct cagtgaagat atcctgcaag gcttctggat acgccttcac taactactgg	840
ctaggttggg taaagcagag gcctggacat ggacttgagt ggattggaga tcttttcct	900
ggaagtggta atactcacta caatgagagg ttcaggggca aagccacact gactgcagac	960

15

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aaatcctcga gcacagcctt tatgcagctc agtagcctga catctgagga ctctgctgtc 1020
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acggtcaccg tctcctcagg tgggtggtggt tctggcggcg gcggctccgg tgggtggtggt 1140
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tcaaggttca gtggcagtggt atctgtgtaca gatttcactc tcaccatcag tagcctggag 1380
cctgaagatt ttgcaatgta ttactgtcaa cagcataatg aatatccgta cacgttcgga 1440
ggggggacca agcttgagat caaacatcat caccatcatc attag 1485

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&lt;210&gt; 12

&lt;211&gt; 494

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; CD3 VHVL stL x 3-1 VHVL

&lt;400&gt; 12

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala  
1 5 10 15

Ser Val Lys Met Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Arg Tyr  
20 25 30

Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile  
35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe  
50 55 60

Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr  
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys  
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly  
100 105 110

Thr Thr Leu Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly  
115 120 125

Ser Gly Gly Gly Gly Ser Asp Ile Gln Leu Thr Gln Ser Pro Ala Ile

130                                      135                                      16                                      140  
 Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser  
 145                                      150                                      155                                      160  
 Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser  
 165                                      170                                      175  
 Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser Gly Val Pro  
 180                                      185                                      190  
 Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile  
 195                                      200                                      205  
 Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp  
 210                                      215                                      220  
 Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys  
 225                                      230                                      235                                      240  
 Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln Ser Gly Ala  
 245                                      250                                      255  
 Glu Leu Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser  
 260                                      265                                      270  
 Gly Tyr Ala Phe Thr Asn Tyr Trp Leu Gly Trp Val Lys Gln Arg Pro  
 275                                      280                                      285  
 Gly His Gly Leu Glu Trp Ile Gly Asp Leu Phe Pro Gly Ser Gly Asn  
 290                                      295                                      300  
 Thr His Tyr Asn Glu Arg Phe Arg Gly Lys Ala Thr Leu Thr Ala Asp  
 305                                      310                                      315                                      320  
 Lys Ser Ser Ser Thr Ala Phe Met Gln Leu Ser Ser Leu Thr Ser Glu  
 325                                      330                                      335  
 Asp Ser Ala Val Tyr Phe Cys Ala Arg Leu Arg Asn Trp Asp Glu Ala  
 340                                      345                                      350  
 Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly  
 355                                      360                                      365  
 Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Leu Val  
 370                                      375                                      380  
 Met Thr Gln Ser Pro Ser Tyr Leu Ala Ala Ser Pro Gly Glu Thr Ile  
 385                                      390                                      395                                      400  
 Thr Ile Asn Cys Arg Ala Ser Lys Ser Ile Ser Lys Tyr Leu Ala Trp  
 405                                      410                                      415



Tyr Gln Glu Lys Pro Gly Lys Thr Asn Lys Leu Leu Ile Tyr Ser Gly  
 420 425 430

Ser Thr Leu Gln Ser Gly Ile Pro Ser Arg Phe Ser Gly Ser Gly Ser  
 435 440 445

Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu Asp Phe  
 450 455 460

Ala Met Tyr Tyr Cys Gln Gln His Asn Glu Tyr Pro Tyr Thr Phe Gly  
 465 470 475 480

Gly Gly Thr Lys Leu Glu Ile Lys His His His His His  
 485 490

<210> 13

<211> 1512

<212> DNA

<213> artificial sequence

<220>

<223> CD3 VHVL stL x 4-7 VHVL

<400> 13

gatatcaaac tgcagcagtc aggggctgaa ctggcaagac ctggggcctc agtgaagatg	60
tcctgcaaga cttctggcta cacctttact aggtacacga tgcactgggt aaaacagagg	120
cctggacagg gtctggaatg gattggatac attaatccta gccgtgggta tactaattac	180
aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac	240
atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat	300
gatgatcatt actgccttga ctactggggc caaggcacca ctctcacagt ctcctcaggt	360
ggtggtgggt ctggcggcgg cggctccggt ggtgggtgggt ctgacattca gctgaccag	420
tctccagcaa tcatgtctgc atctccaggg gagaagggtca ccatgacctg cagagccagt	480
tcaagtgtaa gttacatgaa ctggtaccag cagaagtcag gcacctcccc caaaagatgg	540
atztatgaca catccaaagt ggcttctgga gtcccttata gcttcagtgg cagtgggtct	600
gggacctcat actctctcac aatcagcagc atggaggctg aagatgctgc cacttattac	660
tgccaacagt ggagtagtaa cccgctcacg ttcggtgctg ggaccaagct ggagctgaaa	720
tccggagggt gtggatccga ggtgcagctg ctcgagcagt ctggagctga gctggcgagg	780
cctggggctt cagtgaagct gtcctgcaag gcttctggct acaccttcac aaactatggt	840
ttaagctggg tgaagcagag gcctggacag gtccttgagt ggattggaga ggtttatcct	900
agaattggta atgcttacta caatgagaag ttcaagggca aggccacact gactgcagac	960

18

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aaatcctcca gcacagcgtc catggagctc cgcagcctga cctctgagga ctctgcggtc 1020
tatttctgtg caagacgggg atcctacgat actaactacg actggtactt cgatgtctgg 1080
ggccaagggg ccacgggtcac cgtctcctca ggtggtggtg gttctggcgg cggcggctcc 1140
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catcatcatt ag 1512

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&lt;210&gt; 14

&lt;211&gt; 503

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; CD3 VHVL stL x 4-7 VHVL

&lt;400&gt; 14

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala  
 1 5 10 15

Ser Val Lys Met Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Arg Tyr  
 20 25 30

Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile  
 35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe  
 50 55 60

Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr  
 65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys  
 85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly  
 100 105 110

Thr Thr Leu Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly  
 115 120 125

Ser Gly Gly Gly Gly Ser Asp Ile Gln Leu Thr<sup>19</sup> Gln Ser Pro Ala Ile  
 130 135 140  
 Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser  
 145 150 155 160  
 Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser  
 165 170 175  
 Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser Gly Val Pro  
 180 185 190  
 Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile  
 195 200 205  
 Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp  
 210 215 220  
 Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys  
 225 230 235 240  
 Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln Ser Gly Ala  
 245 250 255  
 Glu Leu Ala Arg Pro Gly Ala Ser Val Lys Leu Ser Cys Lys Ala Ser  
 260 265 270  
 Gly Tyr Thr Phe Thr Asn Tyr Gly Leu Ser Trp Val Lys Gln Arg Pro  
 275 280 285  
 Gly Gln Val Leu Glu Trp Ile Gly Glu Val Tyr Pro Arg Ile Gly Asn  
 290 295 300  
 Ala Tyr Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp  
 305 310 315 320  
 Lys Ser Ser Ser Thr Ala Ser Met Glu Leu Arg Ser Leu Thr Ser Glu  
 325 330 335  
 Asp Ser Ala Val Tyr Phe Cys Ala Arg Arg Gly Ser Tyr Asp Thr Asn  
 340 345 350  
 Tyr Asp Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val  
 355 360 365  
 Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly  
 370 375 380  
 Ser Glu Leu Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu  
 385 390 395 400  
 Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His

20

405                      410                      415

Ser Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln  
                     420                      425                      430

Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val  
                     435                      440                      445

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys  
                     450                      455                      460

Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln  
                     465                      470                      475                      480

Ser Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile  
                     485                      490                      495

Lys His His His His His His  
                     500

&lt;210&gt; 15

&lt;211&gt; 1512

&lt;212&gt; DNA

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; CD3 VHVL stL x 4-7 VLVH

&lt;400&gt; 15

gatatcaaac tgcagcagtc aggggctgaa ctggcaagac ctggggcctc agtgaagatg	60
tcctgcaaga cttctggcta cacctttact aggtacacga tgcactgggt aaaacagagg	120
cctggacagg gtctggaatg gattggatac attaattcta gccgtgggtta tactaattac	180
aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac	240
atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat	300
gatgatcatt actgccttga ctactggggc caaggcacca ctctcacagt ctcctcaggt	360
gggtggtggt ctggcggcgg cggtccgggt ggtggtggt ctgacattca gctgaccag	420
tctccagcaa tcatgtctgc atctccaggg gagaagggtca ccatgacctg cagagccagt	480
tcaagtgtaa gttacatgaa ctggtaccag cagaagtcag gcacctcccc caaaagatgg	540
atttatgaca catccaaagt ggcttctgga gtcccttata gcttcagtgg cagtgggtct	600
gggacctcat actctctcac aatcagcagc atggaggctg aagatgctgc cacttattac	660
tgccaacagt ggagtagtaa cccgctcacg ttcgggtgctg ggaccaagct ggagctgaaa	720
tccggagggtg gtggatccga gctcgtgatg acccagactc cactctccct gcctgtcagt	780
cttgagatc aagcctccat ctcttcgaga tctagtcaga gccttgatca cagtaatgga	840

21

```

aacacctatt tacattggta cctgcagaag ccaggccagt ctccaaagct cctgatctac 900
aaagtttcca accgattttc tgggggtcca gacaggttca gtggcagtgg atcagggaca 960
gatttcacac tcaagatcag cagagtggag gctgaggatc tgggagttta tttctgctct 1020
caaagtacac atgttccgta cacgttcgga ggggggacca agcttgagat caaagggtgg 1080
ggtgggttctg gcggcggcgg ctccggtggt ggtgggttctg aggtgcagct gctcgagcag 1140
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aaggccacac tgactgcaga caaatcctcc agcacagcgt ccatggagct ccgcagcctg 1380
acctctgagg actctgcggt ctatttctgt gcaagacggg gatcctacga tactaactac 1440
gactgggtact tcgatgtctg gggccaaggg accacggtca ccgtctcctc acatcatcac 1500
catcatcatt ag 1512

```

&lt;210&gt; 16

&lt;211&gt; 503

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; CD3 VHVL stL x 4-7 VLVH

&lt;400&gt; 16

```

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
1           5           10           15

```

```

Ser Val Lys Met Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Arg Tyr
20           25           30

```

```

Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35           40           45

```

```

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe
50           55           60

```

```

Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr
65           70           75           80

```

```

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85           90           95

```

```

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100           105           110

```

22

Thr Thr Leu Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly  
 115 120 125  
 Ser Gly Gly Gly Gly Ser Asp Ile Gln Leu Thr Gln Ser Pro Ala Ile  
 130 135 140  
 Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser  
 145 150 155 160  
 Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser  
 165 170 175  
 Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser Gly Val Pro  
 180 185 190  
 Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile  
 195 200 205  
 Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp  
 210 215 220  
 Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys  
 225 230 235 240  
 Ser Gly Gly Gly Gly Ser Glu Leu Val Met Thr Gln Thr Pro Leu Ser  
 245 250 255  
 Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser  
 260 265 270  
 Gln Ser Leu Val His Ser Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu  
 275 280 285  
 Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn  
 290 295 300  
 Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr  
 305 310 315 320  
 Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val  
 325 330 335  
 Tyr Phe Cys Ser Gln Ser Thr His Val Pro Tyr Thr Phe Gly Gly Gly  
 340 345 350  
 Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
 355 360 365  
 Gly Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln Ser Gly Ala Glu  
 370 375 380

Leu Ala Arg Pro Gly Ala Ser Val Lys Leu Ser Cys Lys Ala Ser Gly  
 385 390 395 400

Tyr Thr Phe Thr Asn Tyr Gly Leu Ser Trp Val Lys Gln Arg Pro Gly  
 405 410 415

Gln Val Leu Glu Trp Ile Gly Glu Val Tyr Pro Arg Ile Gly Asn Ala  
 420 425 430

Tyr Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys  
 435 440 445

Ser Ser Ser Thr Ala Ser Met Glu Leu Arg Ser Leu Thr Ser Glu Asp  
 450 455 460

Ser Ala Val Tyr Phe Cys Ala Arg Arg Gly Ser Tyr Asp Thr Asn Tyr  
 465 470 475 480

Asp Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser  
 485 490 495

Ser His His His His His His  
 500

<210> 17

<211> 1503

<212> DNA

<213> artificial sequence

<220>

<223> CD3 VHVL stL x 5-10 VHVL

<400> 17  
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 tcctgcaaga cttctggcta cacctttact aggtacacga tgcactgggt aaaacagagg 120  
 cctggacagg gtctggaatg gattggatac attaataccta gccgtgggta tactaattac 180  
 aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac 240  
 atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat 300  
 gatgatcatt actgccttga ctactggggc caaggcacca ctctcacagt ctctcaggt 360  
 ggtggtggtt ctggcggcgg cggctccggt ggtggtggtt ctgacattca gctgaccag 420  
 tctccagcaa tcatgtctgc atctccaggg gagaaggta ccatgacctg cagagccagt 480  
 tcaagtgtaa gttacatgaa ctggtaccag cagaagtcag gcacctccc caaagatgg 540  
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 gggacctcat actctctcac aatcagcagc atggaggctg aagatgctgc cacttattac 660

24

tgccaacagt ggagtagtaa cccgctcacg ttcggtgctg ggaccaagct ggagctgaaa	720
tccggaggtg gtggatccga ggtgcagctg ctcgagcagt ctggagctga gctggtaagg	780
cctgggactt cagtgaagat atcctgcaag gcttctggat acgccttcac taactactgg	840
ctaggttggg taaagcagag gcctggacat ggacttgagt ggattggaga tattttccct	900
ggaagtggta atatccacta caatgagaag ttcaagggca aagccacact gactgcagac	960
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tatttctgtg caagactgag gaactgggac gagcctatgg actactgggg ccaagggacc	1080
acggtcaccg tctcctcagg tgggtggtgt tctggcggcg gcggctccgg tgggtggtggt	1140
tctgagctcg tgatgacaca gtctccatcc tccctgactg tgacagcagg agagaaggtc	1200
actatgagct gcaagtccag tcagagtctg ttaaacagtg gaaatcaaaa gaactacttg	1260
acctggtacc agcagaaacc agggcagcct cctaaactgt tgatctactg ggcatccact	1320
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accatcagca gtgtgcaggc tgaagacctg gcagtttatt actgtcagaa tgattatagt	1440
tatccgctca cgttcggtgc tgggaccaag cttgagatca aacatcatca ccatcatcat	1500
tag	1503

&lt;210&gt; 18

&lt;211&gt; 500

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; CD3 VHVL stL x 5-10 VHVL

&lt;400&gt; 18

Asp	Ile	Lys	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Leu	Ala	Arg	Pro	Gly	Ala
1				5					10					15	

Ser	Val	Lys	Met	Ser	Cys	Lys	Thr	Ser	Gly	Tyr	Thr	Phe	Thr	Arg	Tyr
			20					25					30		

Thr	Met	His	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile
		35					40					45			

Gly	Tyr	Ile	Asn	Pro	Ser	Arg	Gly	Tyr	Thr	Asn	Tyr	Asn	Gln	Lys	Phe
	50					55					60				

Lys	Asp	Lys	Ala	Thr	Leu	Thr	Thr	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr
65					70					75				80	

Met	Gln	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Tyr	Cys
				85					90					95	



Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly  
 100 105 110  
 Thr Thr Leu Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly  
 115 120 125  
 Ser Gly Gly Gly Gly Ser Asp Ile Gln Leu Thr Gln Ser Pro Ala Ile  
 130 135 140  
 Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser  
 145 150 155 160  
 Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser  
 165 170 175  
 Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser Gly Val Pro  
 180 185 190  
 Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile  
 195 200 205  
 Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp  
 210 215 220  
 Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys  
 225 230 235 240  
 Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln Ser Gly Ala  
 245 250 255  
 Glu Leu Val Arg Pro Gly Thr Ser Val Lys Ile Ser Cys Lys Ala Ser  
 260 265 270  
 Gly Tyr Ala Phe Thr Asn Tyr Trp Leu Gly Trp Val Lys Gln Arg Pro  
 275 280 285  
 Gly His Gly Leu Glu Trp Ile Gly Asp Ile Phe Pro Gly Ser Gly Asn  
 290 295 300  
 Ile His Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp  
 305 310 315 320  
 Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Phe Glu  
 325 330 335  
 Asp Ser Ala Val Tyr Phe Cys Ala Arg Leu Arg Asn Trp Asp Glu Pro  
 340 345 350  
 Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly  
 355 360 365

26

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Leu Val  
 370 375 380

Met Thr Gln Ser Pro Ser Ser Leu Thr Val Thr Ala Gly Glu Lys Val  
 385 390 395 400

Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser Gly Asn Gln  
 405 410 415

Lys Asn Tyr Leu Thr Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys  
 420 425 430

Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val Pro Asp Arg  
 435 440 445

Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser  
 450 455 460

Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn Asp Tyr Ser  
 465 470 475 480

Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Ile Lys His His  
 485 490 495

His His His His  
 500

<210> 19

<211> 1503

<212> DNA

<213> artificial sequence

<220>

<223> CD3 VHVL stL x 5-10 VLVH

<400> 19  
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 cctggacagg gtctggaatg gattggatac attaatccta gccgtgggta tactaattac 180  
 aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac 240  
 atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat 300  
 gatgatcatt actgccttga ctactggggc caaggcacca ctctcacagt ctctcaggt 360  
 ggtggtggtt ctggcggcgg cggtccgggt ggtggtggtt ctgacattca gctgaccag 420  
 tctccagcaa tcatgtctgc atctccaggg gagaagggtca ccatgacctg cagagccagt 480  
 tcaagtgtaa gttacatgaa ctggtaccag cagaagtcag gcacctcccc caaagatgg 540

27

```

atztatgaca catccaaagt ggcttctgga gtcccttatc gcttcagtgg cagtgggtct 600
gggacctcat actctctcac aatcagcagc atggaggctg aagatgctgc cacttattac 660
tgccaacagt ggagtagtaa cccgctcacg ttcggtgctg ggaccaagct ggagctgaaa 720
tccggagggtg gtggatccga gctcgtgatg acacagtctc catcctccct gactgtgaca 780
gcaggagaga aggtcactat gagctgcaag tccagtcaga gtctgttaaa cagtggaaat 840
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ctgacatttg aggactctgc tgtctatttc tgtgcaagac tgaggaactg ggacgagcct 1440
atggactact ggggccaagg gaccacgggc accgtctcct cacatcatca ccatcatcat 1500
tag 1503

```

&lt;210&gt; 20

&lt;211&gt; 500

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; CD3 VHVL stL x 5-10 VLVH

&lt;400&gt; 20

```

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
1           5           10           15

```

```

Ser Val Lys Met Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Arg Tyr
20           25           30

```

```

Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35           40           45

```

```

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe
50           55           60

```

```

Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr

```

65	70	75	80
Met Gln Leu Ser Ser 85 Leu Thr Ser Glu Asp 90 Ser Ala Val Tyr Tyr 95 Cys			
Ala Arg Tyr Tyr 100 Asp Asp His Tyr Cys 105 Leu Asp Tyr Trp Gly 110 Gln Gly			
Thr Thr Leu 115 Thr Val Ser Ser Gly 120 Gly Gly Gly Ser Gly 125 Gly Gly Gly			
Ser Gly 130 Gly Gly Gly Ser Asp 135 Ile Gln Leu Thr Gln 140 Ser Pro Ala Ile			
Met 145 Ser Ala Ser Pro Gly 150 Glu Lys Val Thr Met 155 Thr Cys Arg Ala Ser 160			
Ser Ser Val Ser Tyr 165 Met Asn Trp Tyr Gln 170 Gln Lys Ser Gly Thr 175 Ser			
Pro Lys Arg Trp 180 Ile Tyr Asp Thr Ser 185 Lys Val Ala Ser Gly 190 Val Pro			
Tyr Arg Phe 195 Ser Gly Ser Gly Ser 200 Gly Thr Ser Tyr Ser 205 Leu Thr Ile			
Ser Ser 210 Met Glu Ala Glu Asp 215 Ala Ala Thr Tyr Tyr 220 Cys Gln Gln Trp			
Ser 225 Ser Asn Pro Leu Thr 230 Phe Gly Ala Gly Thr 235 Lys Leu Glu Leu Lys 240			
Ser Gly Gly Gly Gly 245 Ser Glu Leu Val Met 250 Thr Gln Ser Pro Ser 255			
Leu Thr Val Thr 260 Ala Gly Glu Lys Val 265 Thr Met Ser Cys Lys 270 Ser Ser			
Gln Ser Leu 275 Leu Asn Ser Gly Asn 280 Gln Lys Asn Tyr Leu 285 Thr Trp Tyr			
Gln Gln Lys Pro Gly Gln Pro 295 Pro Lys Leu Leu Ile 300 Tyr Trp Ala Ser			
Thr Arg Glu Ser Gly Val 310 Pro Asp Arg Phe Thr 315 Gly Ser Gly Ser Gly 320 )			
Thr Asp Phe Thr Leu 325 Thr Ile Ser Ser Val 330 Gln Ala Glu Asp Leu 335 Ala			
Val Tyr Tyr Cys 340 Gln Asn Asp Tyr Ser 345 Tyr Pro Leu Thr Phe 350 Gly Ala			

29

Gly Thr Lys<sub>355</sub> Leu Glu Ile Lys Gly<sub>360</sub> Gly Gly Gly Ser Gly<sub>365</sub> Gly Gly Gly

Ser Gly<sub>370</sub> Gly Gly Gly Ser Glu<sub>375</sub> Val Gln Leu Leu Glu<sub>380</sub> Gln Ser Gly Ala

Glu<sub>385</sub> Leu Val Arg Pro Gly<sub>390</sub> Thr Ser Val Lys Ile<sub>395</sub> Ser Cys Lys Ala Ser<sub>400</sub>

Gly Tyr Ala Phe Thr<sub>405</sub> Asn Tyr Trp Leu Gly<sub>410</sub> Trp Val Lys Gln Arg Pro<sub>415</sub>

Gly His Gly Leu<sub>420</sub> Glu Trp Ile Gly Asp<sub>425</sub> Ile Phe Pro Gly Ser<sub>430</sub> Gly Asn

Ile His Tyr<sub>435</sub> Asn Glu Lys Phe Lys<sub>440</sub> Gly Lys Ala Thr Leu Thr Ala Asp<sub>445</sub>

Lys Ser Ser Ser Thr Ala Tyr<sub>455</sub> Met Gln Leu Ser Ser<sub>460</sub> Leu Thr Phe Glu

Asp Ser Ala Val Tyr Phe<sub>470</sub> Cys Ala Arg Leu Arg<sub>475</sub> Asn Trp Asp Glu Pro<sub>480</sub>

Met Asp Tyr Trp Gly<sub>485</sub> Gln Gly Thr Thr Val Thr Val Ser Ser His<sub>495</sub> His

His His His His<sub>500</sub>

<210> 21

<211> 57

<212> DNA

<213> artificial sequence

<220>

<223> 3' CD3 VH GS15 primer

<400> 21  
ggagccgcccg ccgccagaac caccaccacc tgaggagact gtgagagtgg tgccttg

57

<210> 22

<211> 53

<212> DNA

<213> artificial sequence

<220>

<223> 5' CD3 VL GS15 primer

<400> 22

ggcggcggcg gctccggtgg tggagggttct gacattcagc tgacccagtc tcc 53

<210> 23

<211> 51

<212> DNA

<213> artificial sequence

<220>

<223> 4-7 VH GS15 FOR

<400> 23

ggcggcggcg gctccggtgg tggagggttct gaggtgcagc tgctcgagca g 51

<210> 24

<211> 53

<212> DNA

<213> artificial sequence

<220>

<223> 4-7 VH SalI REV primer

<400> 24

ttttaagtcg acctaagatgat gatgatgatg atgtgaggag acggtgaccg tgg 53

<210> 25

<211> 49

<212> DNA

<213> artificial sequence

<220>

<223> 5-10 VLBSpEI38 primer

<400> 25

ctgaaatccg gaggtggtgg atccgagctc gtgatgacac agtctccat 49

<210> 26

<211> 53

<212> DNA

31

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; 5-10 VLGS15REV primer

&lt;400&gt; 26

ggagccgccc ccgccagaac caccaccacc ttgatctca agcttggtcc cag

53

&lt;210&gt; 27

&lt;211&gt; 49

&lt;212&gt; DNA

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; 5-10 VH GS15 FOR primer

&lt;400&gt; 27

ggcggcgggc gctccggtgg tgggtggttct gaggtgcagc tgctcgagc

49

&lt;210&gt; 28

&lt;211&gt; 53

&lt;212&gt; DNA

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; 5-10 VHSa1REV primer

&lt;400&gt; 28

ttttaagtgc acctaattgat gatgatgatg atgtgaggag acggtgaccg tgg

53

&lt;210&gt; 29

&lt;211&gt; 1581

&lt;212&gt; DNA

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; 3-5(VL-VH)xanti-CD3

&lt;400&gt; 29

atgggatgga gctgtatcat cctcttcttg gtagcaacag ctacaggtgt aactccgcg

60

cgcgagctcg tgatgacca gactccactc tccctgcctg tcagtcttgg agatcaagcc

120

tccatctctt gcagatctag tcagagcctt gtacacagta atggaaacac ctatttacat

180

32

tggtacctgc agaagccagg ccagtcctcca aagctcctga tctacaaagt ttccaaccga	240
ttttctgggg tcccagacag gttcagtggc agtgatcag ggacagattt cacactcaag	300
atcagcagag tggaggctga ggatctggga gtttatttct gctctcaaag tacacatggt	360
ccgtacacgt tcggaggggg gaccaagctt gagatcaaag gtggtggtgg ttctggcggc	420
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gtaaggcctg ggacttcagt gaagctgtcc tgcaaggctt ctggctacac cttcacaagc	540
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tatcctagaa ttggtaatgc ttactacaat gagaagttca agggcaaggc cacactgact	660
gcagacaaat cctccagcac agcgtccatg gagctccgca gcctgacatc tgaggactct	720
gcggtctatt tctgtgcaag acggggatcc tacggtagta actacgactg gtacttcgat	780
gtctggggcc aagggaccac ggtcaccgtc tcctcggag gtggtggatc cgatatcaaa	840
ctgcagcagt caggggctga actggcaaga cctggggcct cagtgaagat gtcctgcaag	900
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ggtctggaat ggattggata cattaatcct agccgtgggt atactaatta caatcagaag	1020
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agcagcctga catctgagga ctctgcagtc tattactgtg caagatatta tgatgatcat	1140
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catcatcacc atcatcatta g	1581

&lt;210&gt; 30

&lt;211&gt; 526

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; 3-5(VL-VH)xanti-CD3

&lt;400&gt; 30

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly  
 1 5 10 15



Val His Ser Ala Arg Glu Leu Val Met Thr <sup>33</sup> Gln Thr Pro Leu Ser Leu  
                     20                    25                    30

Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln  
                     35                    40                    45

Ser Leu Val His Ser Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln  
                     50                    55                    60

Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg  
                     65                    70                    75                    80

Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp  
                     85                    90                    95

Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr  
                     100                    105                    110

Phe Cys Ser Gln Ser Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr  
                     115                    120                    125

Lys Leu Glu Ile Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly  
                     130                    135                    140

Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln Ser Gly Ala Glu Leu  
                     145                    150                    155                    160

Val Arg Pro Gly Thr Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr  
                     165                    170                    175

Thr Phe Thr Ser Tyr Gly Leu Ser Trp Val Lys Gln Arg Thr Gly Gln  
                     180                    185                    190

Gly Leu Glu Trp Ile Gly Glu Val Tyr Pro Arg Ile Gly Asn Ala Tyr  
                     195                    200                    205

Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser  
                     210                    215                    220

Ser Ser Thr Ala Ser Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser  
                     225                    230                    235                    240

Ala Val Tyr Phe Cys Ala Arg Arg Gly Ser Tyr Gly Ser Asn Tyr Asp  
                     245                    250                    255

Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser  
                     260                    265                    270

Gly Gly Gly Gly Ser Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu  
                     275                    280                    285

Ala Arg Pro Gly Ala Ser Val Lys Met Ser Cys Lys Thr Ser Gly Tyr

290 295 34 300  
 Thr Phe Thr Arg Tyr Thr Met His Trp Val Lys Gln Arg Pro Gly Gln  
 305 310 315 320  
 Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn  
 325 330 335  
 Tyr Asn Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser  
 340 345 350  
 Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser  
 355 360 365  
 Ala Val Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp  
 370 375 380  
 Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Val Glu Gly Gly  
 385 390 395 400  
 Ser Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Val Asp Asp Ile  
 405 410 415  
 Gln Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys  
 420 425 430  
 Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Met Asn Trp  
 435 440 445  
 Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr  
 450 455 460  
 Ser Lys Val Ala Ser Gly Val Pro Tyr Arg Phe Ser Gly Ser Gly Ser  
 465 470 475 480  
 Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala  
 485 490 495  
 Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly  
 500 505 510  
 Ala Gly Thr Lys Leu Glu Leu Lys His His His His His His  
 515 520 525

&lt;210&gt; 31

&lt;211&gt; 40

&lt;212&gt; DNA

&lt;213&gt; artificial sequence

35

&lt;220&gt;

&lt;223&gt; Me81 primer

&lt;400&gt; 31

ggatgcgcgc gagctcgtga tgacccagac tccactctcc

40

&lt;210&gt; 32

&lt;211&gt; 60

&lt;212&gt; DNA

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; Me83 primer

&lt;400&gt; 32

ggttctggcg gcggcggctc cgggtggtggt ggttctgagg tgcagctgct cgacagtctg 60

&lt;210&gt; 33

&lt;211&gt; 41

&lt;212&gt; DNA

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; Me84 primer

&lt;400&gt; 33

gtgctccgga ggagacggtg accgtggtcc cttggcccca g 41

&lt;210&gt; 34

&lt;211&gt; 53

&lt;212&gt; DNA

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; Me90 primer

&lt;400&gt; 34

ccggagccgc cgccgccaga accaccacca cctttgatct caagcttggt ccc 53

&lt;210&gt; 35

&lt;211&gt; 1548

&lt;212&gt; DNA

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; 3-1(VLVH)xanti-CD3

&lt;400&gt; 35

atgggatgga gctgtatcat cctcttcttg gtagcaacag ctacaggtgt acactccgag	60
ctcgtcatga cccagtctcc atcttatctt gctgcatctc ctggagaaac cattactatt	120
aattgcaggg caagtaagag cattagcaaa tathtagcct ggtatcaaga gaaacctggg	180
aaaactaata agcttcttat ctactctgga tccactttgc aatctggaat tccatcaagg	240
ttcagtggca gtggatctgg tacagatttc actctacca tcagtagcct ggagcctgaa	300
gattttgcaa tgtattactg tcaacagcat aatgaatatc cgtacacgtt cggagggggg	360
accaagcttg agatcaaagg tgggtgggtg tctggcggcg gcggctccgg tgggtgggtg	420
tctgaggtgc agctgctcga gcagtctgga gctgagctgg tgaaacctgg ggcctcagt	480
aagatatcct gcaaggcttc tggatacgcc ttcactaact actggctagg ttgggtaaag	540
cagaggcctg gacatggact tgagtggatt ggagatcttt tccctggaag tggtaatact	600
cactacaatg agaggttcag gggcaaagcc aactgactg cagacaaatc ctcgagcaca	660
gcctttatgc agctcagtag cctgacatct gaggactctg ctgtctatct ctgtgcaaga	720
ttgaggaact gggacgaggc tatggactac tggggccaag ggaccacggt caccgtctcc	780
tccggagggtg gtggatccga tatcaaactg cagcagtcag gggctgaact ggcaagacct	840
ggggcctcag tgaagatgtc ctgcaagact tctggctaca cttttactag gtacacgatg	900
cactgggtaa aacagaggcc tggacagggt ctggaatgga ttggatacat taatcctagc	960
cgtggttata ctaattacaa tcagaagttc aaggacaagg ccacattgac tacagacaaa	1020
tcctccagca cagcctacat gcaactgagc agcctgacat ctgaggactc tgcagtctat	1080
tactgtgcaa gatattatga tgatcattac tgccttgact actggggcca aggcaccact	1140
ctcacagtct cctcagtcga aggtggaagt ggaggttctg gtggaagtgg aggttcaggt	1200
ggagtgcagc acattcagct gaccagctct ccagcaatca tgtctgcac tccagggggag	1260
aaggtcacca tgacctgcag agccagttca agtgtaagtt acatgaactg gtaccagcag	1320
aagtcaggca cctcccccaa aagatggatt tatgacacat ccaaagtggc ttctggagtc	1380
ccttatcgct tcagtggcag tgggtctggg acctcact ctctcacaat cagcagcatg	1440
gaggctgaag atgctgccac ttatractgc caacagtgga gtagtaacc gctcacgttc	1500
ggtgctggga ccaagctgga gctgaaacat catcaccatc atcattag	1548

&lt;210&gt; 36

&lt;211&gt; 515

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; 3-1(VLVH)xanti-CD3

&lt;400&gt; 36

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly  
 1 5 10 15

Val His Ser Glu Leu Val Met Thr Gln Ser Pro Ser Tyr Leu Ala Ala  
 20 25 30

Ser Pro Gly Glu Thr Ile Thr Ile Asn Cys Arg Ala Ser Lys Ser Ile  
 35 40 45

Ser Lys Tyr Leu Ala Trp Tyr Gln Glu Lys Pro Gly Lys Thr Asn Lys  
 50 55 60

Leu Leu Ile Tyr Ser Gly Ser Thr Leu Gln Ser Gly Ile Pro Ser Arg  
 65 70 75 80

Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser  
 85 90 95

Leu Glu Pro Glu Asp Phe Ala Met Tyr Tyr Cys Gln Gln His Asn Glu  
 100 105 110

Tyr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Gly Gly  
 115 120 125

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val Gln  
 130 135 140

Leu Leu Glu Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala Ser Val  
 145 150 155 160

Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Thr Asn Tyr Trp Leu  
 165 170 175

Gly Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp Ile Gly Asp  
 180 185 190

Leu Phe Pro Gly Ser Gly Asn Thr His Tyr Asn Glu Arg Phe Arg Gly  
 195 200 205

Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Phe Met Gln  
 210 215 220

Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg  
 225 230 235 240

Leu Arg Asn Trp Asp Glu Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr

245 250 38 255  
 Val Thr Val Ser Ser Gly Gly Gly Gly Ser Asp Ile Lys Leu Gln Gln  
 260 265 270  
 Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala Ser Val Lys Met Ser Cys  
 275 280 285  
 Lys Thr Ser Gly Tyr Thr Phe Thr Arg Tyr Thr Met His Trp Val Lys  
 290 295 300  
 Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser  
 305 310 315 320  
 Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Asp Lys Ala Thr Leu  
 325 330 335  
 Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu  
 340 345 350  
 Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp  
 355 360 365  
 His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser  
 370 375 380  
 Ser Val Glu Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly  
 385 390 395 400  
 Gly Val Asp Asp Ile Gln Leu Thr Gln Ser Pro Ala Ile Met Ser Ala  
 405 410 415  
 Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val  
 420 425 430  
 Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg  
 435 440 445  
 Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser Gly Val Pro Tyr Arg Phe  
 450 455 460  
 Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met  
 465 470 475 480  
 Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn  
 485 490 495  
 Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys His His His  
 500 505 510  
 His His His  
 515

&lt;210&gt; 37

&lt;211&gt; 52

&lt;212&gt; DNA

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; Me91a primer

&lt;400&gt; 37

ggattgtaca ctccgagctc gtcattgaccc agtctccatc ttatcttgct gc 52

&lt;210&gt; 38

&lt;211&gt; 1566

&lt;212&gt; DNA

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; 4-1(VLVH)xanti-CD3

&lt;400&gt; 38

atgggatgga gctgtatcat cctcttcttg gtagcaacag ctacaggtgt acactccgag 60

ctcgtgatga cacagtctcc atcctccctg agtgtgtcag caggagagaa ggtcactatg 120

agctgcaagt ccagtcagag tctgttaaag agtggaatc aaaagaacta cttggcctgg 180

taccagcaga aaccagggga gcctcctaaa ctgttgatct acggggcatc cactagggaa 240

tctgggggcc ctgatcgctt cacaggcagt ggatctggaa cagatttcac tctcaccatc 300

agcagtgtgc aggctgaaga cctggcagtt tattactgtc agaatgatta tagttatccg 360

tacacgttcg gaggggggac caagcttgag atcaaagggt gtggtggttc tggcggcggc 420

ggctccggtg gtggtggttc tgagggtcag ctgctcgagc agtctggagc tgagctggtg 480

aggcctggga cttcagtga gatatcctgc aaggcttctg gatacgctt cactaactac 540

tggctaggtt gggttaagca gaggcctgga catggacttg aatgggttgg agatattttc 600

cctggaagtg gtaatgtcga ctacaatgag aagttcaagg gcaaagccac actgactgca 660

gacaagtctt cgtacacagc ctatatgcag ctcatgagcc tgacatctga ggactctgct 720

gtctattttt gtgcaagatt gcggaactgg gacgaggcta tggactactg gggccaaggg 780

accacggtca ccgtctctc cggaggtggg ggatccgata tcaaactgca gcagtcaggg 840

gctgaactgg caagacctgg ggcctcagtg aagatgtcct gcaagacttc tggctacacc 900

tttactaggt acacgatgca ctgggtaaaa cagaggcctg gacagggctt ggaatggatt 960

ggatacatca atcctagccg tggttatact aattacaatc agaagttcaa ggacaaggcc 1020

40

```

acattgacta cagacaaatc ctccagcaca gcctacatgc aactgagcag cctgacatct 1080
gaggactctg cagtctatta ctgtgcaaga tattatgatg atcattactg ccttgactac 1140
tggggccaag gcaccactct cacagtctcc tcagtcgaag gtggaagtgg aggttctggt 1200
ggaagtggag gttcaggtgg agtcgacgac attcagctga cccagtctcc agcaatcatg 1260
tctgcatctc caggggagaa ggtcaccatg acctgcagag ccagttcaag tgtaagttac 1320
atgaactggg accagcagaa gtcaggcacc tccccaaaa gatggattta tgacacatcc 1380
aaagtggctt ctggagtccc ttatcgcttc agtggcagtg ggtctgggac ctcatactct 1440
ctcacaatca gcagcatgga ggctgaagat gctgccactt attactgcca acagtggagt 1500
agtaaccgcg tcacgttcgg tgctgggacc aagctggagc tgaaacatca tcaccatcat 1560
cattag 1566

```

&lt;210&gt; 39

&lt;211&gt; 521

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; 4-1(VLVH)xanti-CD3

&lt;400&gt; 39

```

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1           5           10           15

```

```

Val His Ser Glu Leu Val Met Thr Gln Ser Pro Ser Ser Leu Ser Val
20           25           30

```

```

Ser Ala Gly Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu
35           40           45

```

```

Leu Asn Ser Gly Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys
50           55           60

```

```

Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg Glu
65           70           75           80

```

```

Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe
85           90           95

```

```

Thr Leu Thr Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr
100          105          110

```

```

Cys Gln Asn Asp Tyr Ser Tyr Pro Tyr Thr Phe Gly Gly Gly Thr Lys
115          120          125

```



Leu Glu Ile Lys Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly  
 130 135 140 41  
 Gly Gly Ser Glu Val Gln Leu Leu Glu Gln Ser Gly Ala Glu Leu Val  
 145 150 155 160  
 Arg Pro Gly Thr Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala  
 165 170 175  
 Phe Thr Asn Tyr Trp Leu Gly Trp Val Lys Gln Arg Pro Gly His Gly  
 180 185 190  
 Leu Glu Trp Val Gly Asp Ile Phe Pro Gly Ser Gly Asn Ala His Tyr  
 195 200 205  
 Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser  
 210 215 220  
 Tyr Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala  
 225 230 235 240  
 Val Tyr Phe Cys Ala Arg Leu Arg Asn Trp Asp Glu Ala Met Asp Tyr  
 245 250 255  
 Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser  
 260 265 270  
 Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala  
 275 280 285  
 Ser Val Lys Met Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Arg Tyr  
 290 295 300  
 Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile  
 305 310 315 320  
 Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe  
 325 330 335  
 Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr  
 340 345 350  
 Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys  
 355 360 365  
 Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly  
 370 375 380  
 Thr Thr Leu Thr Val Ser Ser Val Glu Gly Gly Ser Gly Gly Ser Gly  
 385 390 395 400  
 Gly Ser Gly Gly Ser Gly Gly Val Asp Asp Ile Gln Leu Thr Gln Ser

Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys  
420 425 430

Arg Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser  
435 440 445

Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser  
450 455 460

Gly Val Pro Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser  
465 470 475 480

Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys  
485 490 495

Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu  
500 505 510

Glu Leu Lys His His His His His His  
515 520

<210>	40
<211>	44
<212>	DNA
<213>	artificial sequence

```
<220>
<223> Me92a primer
<400> 40
ggattgtaca ctccgagctc gtgatgacac agtctccatc ctcc
```

44

<210>	41
<211>	1581
<212>	DNA
<213>	artificial sequence

```

<220>
<223> 4-7(VL-VH)xanti CD3
<400> 41
atgggatgga gctgtatcat cctcttcttg gtagcaacag ctacaggtgt acactccgcg      60
cgcgagctcg tgatgaccca gactccactc tccctgcctg tcagtcttgg agatcaagcc      120
tccatctctt gcagatctag tcagagcctt gtacacagta atggaaacac ctattttacat      180

```

43

tggtagctgc agaagccagg ccagctctcca aagctcctga tctacaaagt ttccaaccga 240  
 ttttctgggg tcccagacag gttcagtggc agtggatcag ggacagattt cacactcaag 300  
 atcagcagag tggaggctga ggatctggga gtttatttct gctctcaaag tacacatgtt 360  
 ccgtacacgt tcggaggggg gaccaagctt gagatcaaag gtgggtggtg ttctggcggc 420  
 ggcggctccg gtgggtggtg ttctgaggtg cagctgctcg agcagtctgg agctgagctg 480  
 gcgaggcctg gggcttcagt gaagctgtcc tgcaaggctt ctggctacac cttcacaac 540  
 tatggtttta gctgggtgaa gcagaggcct ggacaggctc ttgagtggat tggagaggtt 600  
 tactctagaa ttggaatgc ttactacaat gagaagttca agggcaaggc cacactgact 660  
 gcagacaaat cctccagcac agcgtccatg gagctccgca gcctgacctc tgaggactct 720  
 gcggtctatt tctgtgcaag acggggatcc tacgatacta actacgactg gtacttcgat 780  
 gtctggggcc aagggaccac ggtcaccgtc tcctccggag gtgggtggatc cgatatcaaa 840  
 ctgcagcagt caggggctga actggcaaga cctggggcct cagtgaagat gtcctgcaag 900  
 acttctggct acacctttac taggtacacg atgactggg taaaacagag gcctggacag 960  
 ggtctggaat ggattggata cattaatcct agccgtgggt atactaatta caatcagaag 1020  
 ttcaaggaca aggccacatt gactacagac aaatcctcca gcacagccta catgcaactg 1080  
 agcagcctga catctgagga ctctgcagtc tattactgtg caagatatta tgatgatcat 1140  
 tactgccttg actactgggg ccaaggcacc actctcacag tctcctcagt cgaagggtgga 1200  
 agtggagggt ctggtggaag tggaggttca ggtggagtcg acgacattca gctgacccag 1260  
 tctccagcaa tcatgtctgc atctccaggg gagaaggcca ccatgacctg cagagccagt 1320  
 tcaagtgtaa gttacatgaa ctggtaccag cagaagtcag gcacctcccc caaaagatgg 1380  
 atttatgaca catccaaagt ggcttctgga gtcccttatc gcttcagtgg cagtgggtct 1440  
 gggacctcat actctctcac aatcagcagc atggaggctg aagatgctgc cacttattac 1500  
 tgccaacagt ggagtagtaa cccgctcacg ttcggtgctg ggaccaagct ggagctgaaa 1560  
 catcatcacc atcatcatta g 1581

&lt;210&gt; 42

&lt;211&gt; 526

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; 4-7(VL-VH)xanti CD3

&lt;400&gt; 42

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly  
 1 5 10 15

44

Val His Ser Ala Arg Glu Leu Val Met Thr Gln Thr Pro Leu Ser Leu  
 20 25 30  
 Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln  
 35 40 45  
 Ser Leu Val His Ser Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln  
 50 55 60  
 Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg  
 65 70 75 80  
 Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp  
 85 90 95  
 Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr  
 100 105 110  
 Phe Cys Ser Gln Ser Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr  
 115 120 125  
 Lys Leu Glu Ile Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly  
 130 135 140  
 Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln Ser Gly Ala Glu Leu  
 145 150 155 160  
 Ala Arg Pro Gly Ala Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr  
 165 170 175  
 Thr Phe Thr Asn Tyr Gly Leu Ser Trp Val Lys Gln Arg Pro Gly Gln  
 180 185 190  
 Val Leu Glu Trp Ile Gly Glu Val Tyr Pro Arg Ile Gly Asn Ala Tyr  
 195 200 205  
 Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser  
 210 215 220  
 Ser Ser Thr Ala Ser Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser  
 225 230 235 240  
 Ala Val Tyr Phe Cys Ala Arg Arg Gly Ser Tyr Asp Thr Asn Tyr Asp  
 245 250 255  
 Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser  
 260 265 270  
 Gly Gly Gly Gly Ser Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu  
 275 280 285

Ala Arg Pro Gly Ala Ser Val Lys Met Ser Cys<sup>45</sup> Lys Thr Ser Gly Tyr  
 290 300  
 Thr Phe Thr Arg Tyr Thr Met His Trp Val Lys Gln Arg Pro Gly Gln  
 305 310 315 320  
 Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn  
 325 330 335  
 Tyr Asn Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser  
 340 345 350  
 Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser  
 355 360 365  
 Ala Val Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp  
 370 375 380  
 Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Val Glu Gly Gly  
 385 390 395 400  
 Ser Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Val Asp Asp Ile  
 405 410 415  
 Gln Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys  
 420 425 430  
 Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Met Asn Trp  
 435 440 445  
 Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr  
 450 455 460  
 Ser Lys Val Ala Ser Gly Val Pro Tyr Arg Phe Ser Gly Ser Gly Ser  
 465 470 475 480  
 Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala  
 485 490 495  
 Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly  
 500 505 510  
 Ala Gly Thr Lys Leu Glu Leu Lys His His His His His His  
 515 520 525

&lt;210&gt; 43

&lt;211&gt; 1566

&lt;212&gt; DNA

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; 5-10(VLVH)xanti-CD3

&lt;400&gt; 43

```

atgggatgga gctgtatcat cctcttcttg gtagcaacag ctacaggtgt aactccgag      60
ctcgtgatga cacagtctcc atcctccctg actgtgacag caggagagaa ggtcactatg    120
agctgcaagt ccagtcagag tctgttaaac agtggaatc aaaagaacta cttgacctgg    180
taccagcaga aaccagggca gcctcctaaa ctgttgatct actgggcac cactagggaa    240
tctgggggcc ctgacgctt cacaggcagt ggatctggaa cagatttcac tctcaccatc    300
agcagtgtgc aggctgaaga cctggcagtt tattactgtc agaatgatta tagttatccg    360
ctcacgttcg gtgctgggac caagcttgag atcaaagggt gtggtgggtc tggcggcggc    420
ggctccgggt gtggtgggtc tgagggtgac ctgctcgagc agtctggagc tgagctggta    480
aggcctggga cttcagtga gatatactgc aaggcttctg gatacgctt cactaactac    540
tggctagggt gggtaaagca gaggcctgga catggacttg agtggattgg agatattttc    600
cctggaagtg gtaatatcca ctacaatgag aagttcaagg gcaaagccac actgactgca    660
gacaaatctt cgagcacagc ctatatgcag ctcatgagcc tgacatttga ggactctgct    720
gtctatttct gtgcaagact gaggaactgg gacgagccta tggactactg gggccaaggg    780
accacgggtc cgtctctctc cggagggtgg ggatccgata tcaaactgca gcagtcaggg    840
gctgaactgg caagacctgg ggcctcagtg aagatgtcct gcaagacttc tggctacacc    900
tttactaggt acacgatgca ctgggtaaaa cagaggcctg gacaggggtc ggaatggatt    960
ggatacatta atcctagccg tggttatact aattacaatc agaagttcaa ggacaaggcc   1020
acattgacta cagacaaatc ctccagcaca gcctacatgc aactgagcag cctgacatct   1080
gaggactctg cagtctatta ctgtgcaaga tattatgatg atcattactg ccttgactac   1140
tggggccaag gcaccactct cacagtctcc tcagtcgaag gtggaagtgg aggttctggt   1200
ggaagtggag gttcagggtg agtcgacgac attcagctga ccagtcctc agcaatcatg   1260
tctgcatctc caggggagaa ggtcaccatg acctgcagag ccagttcaag tgtaagttac   1320
atgaactggg accagcagaa gtcaggcacc tccccaaaa gatggattta tgacacatcc   1380
aaagtggctt ctggagtccc ttatcgcttc agtggcagtg ggtctgggac ctcatctct   1440
ctcacaatca gcagcatgga ggctgaagat gctgccactt attactgcca acagtggagt   1500
agtaaccgcg tcacgttcgg tgctgggacc aagctggagc tgaacatca tcaccatcat   1560
cattag                                           1566

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&lt;210&gt; 44

&lt;211&gt; 521

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; 5-10(VLVH)xanti-CD3

&lt;400&gt; 44

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly  
 1 5 10 15

Val His Ser Glu Leu Val Met Thr Gln Ser Pro Ser Ser Leu Thr Val  
 20 25 30

Thr Ala Gly Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu  
 35 40 45

Leu Asn Ser Gly Asn Gln Lys Asn Tyr Leu Thr Trp Tyr Gln Gln Lys  
 50 55 60

Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu  
 65 70 75 80

Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe  
 85 90 95

Thr Leu Thr Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr  
 100 105 110

Cys Gln Asn Asp Tyr Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys  
 115 120 125

Leu Glu Ile Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly  
 130 135 140

Gly Gly Ser Glu Val Gln Leu Leu Glu Gln Ser Gly Ala Glu Leu Val  
 145 150 155 160

Arg Pro Gly Thr Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala  
 165 170 175

Phe Thr Asn Tyr Trp Leu Gly Trp Val Lys Gln Arg Pro Gly His Gly  
 180 185 190

Leu Glu Trp Ile Gly Asp Ile Phe Pro Gly Ser Gly Asn Ile His Tyr  
 195 200 205

Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser  
 210 215 220

Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Phe Glu Asp Ser Ala  
 225 230 235 240

Val Tyr Phe Cys Ala Arg Leu Arg Asn Trp Asp Glu Pro Met Asp Tyr

245 250<sup>48</sup> 255  
 Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser  
 260 265 270  
 Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala  
 275 280 285  
 Ser Val Lys Met Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Arg Tyr  
 290 295 300  
 Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile  
 305 310 315 320  
 Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe  
 325 330 335  
 Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr  
 340 345 350  
 Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys  
 355 360 365  
 Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly  
 370 375 380  
 Thr Thr Leu Thr Val Ser Ser Val Glu Gly Gly Ser Gly Gly Ser Gly  
 385 390 395 400  
 Gly Ser Gly Gly Ser Gly Gly Val Asp Asp Ile Gln Leu Thr Gln Ser  
 405 410 415  
 Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys  
 420 425 430  
 Arg Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser  
 435 440 445  
 Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser  
 450 455 460  
 Gly Val Pro Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser  
 465 470 475 480  
 Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys  
 485 490 495  
 Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu  
 500 505 510  
 Glu Leu Lys His His His His His His  
 515 520



<210> 45  
 <211> 1494  
 <212> DNA  
 <213> artificial sequence

<220>

<223> CD3 VHVL aL x 3-1 VHVL

<400> 45  
 gatatacaaac tgcagcagtc aggggctgaa ctggcaagac ctggggcctc agtgaagatg 60  
 tcctgcaaga cttctggcta cacctttact aggtacacga tgcactgggt aaaacagagg 120  
 cctggacagg gtctggaatg gattggatac attaatccta gccgtgggtta tactaattac 180  
 aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac 240  
 atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat 300  
 gatgatcatt actgccttga ctactggggc caaggcacca ctctcacagt ctctcagtc 360  
 gaagggtgaa gtggagggtc tgggtggaagt ggagggttcag gtggagtcga cgacattcag 420  
 ctgaccagtc ctccagcaat catgtctgca tctccagggg agaaggtcac catgacctgc 480  
 agagccagtt caagtgtgaa ttacatgaac tgggtaccagc agaagtcagg cacctcccc 540  
 aaaagatgga tttatgacac atccaaagtg gcttctggag tcccttatcg cttcagtggc 600  
 agtgggtctg ggacctcata ctctctcaca atcagcagca tggaggctga agatgctgcc 660  
 acttattact gccaacagtg gagtagtaac ccgctcacgt tcggtgctgg gaccaagctg 720  
 gagctgaaat ccggagggtg tggatccgag gtgcagctgc tcgagcagtc tggagctgag 780  
 ctggtgaaac ctggggcctc agtgaagata tcctgcaagg cttctggata cgccttcact 840  
 aactactggc taggttgggt aaagcagagg cctggacatg gacttgagtg gattggagat 900  
 cttttccctg gaagtggtaa tactcactac aatgagaggt tcaggggcaa agccacactg 960  
 actgcagaca aatcctcgag cacagccttt atgcagctca gtagcctgac atctgaggac 1020  
 tctgctgtct atttctgtgc aagattgagg aactgggacg aggctatgga ctactggggc 1080  
 caagggacca cggtcaccgt ctctcaggt ggtggtggtt ctggcggcgg cggctccggt 1140  
 ggtggtggtt ctgagctcgt catgaccag tctccatctt atcttgctgc atctcctgga 1200  
 gaaaccatta ctattaattg cagggaagt aagagcatta gcaaataatt agcctggtat 1260  
 caagagaaac ctgggaaaac taataagctt cttatctact ctggatccac ttgcaatct 1320  
 ggaattccat caaggttcag tggcagtgga tctggtacag atttactct caccatcagt 1380  
 agcctggagc ctgaagattt tgcaatgtat tactgtcaac agcataatga atatccgtac 1440  
 acgttcggag gggggaccaa gcttgagatc aaacatcatc accatcatca ttag 1494

<210> 46

50

&lt;211&gt; 497

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; CD3 VHVL aL x 3-1 VHVL

&lt;400&gt; 46

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala  
 1 5 10 15

Ser Val Lys Met Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Arg Tyr  
 20 25 30

Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile  
 35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe  
 50 55 60

Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr  
 65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys  
 85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly  
 100 105 110

Thr Thr Leu Thr Val Ser Ser Val Glu Gly Gly Ser Gly Gly Ser Gly  
 115 120 125

Gly Ser Gly Gly Ser Gly Gly Val Asp Asp Ile Gln Leu Thr Gln Ser  
 130 135 140

Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys  
 145 150 155 160

Arg Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser  
 165 170 175

Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser  
 180 185 190

Gly Val Pro Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser  
 195 200 205

Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys  
 210 215 220

51

Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu  
 225 230 235 240  
 Glu Leu Lys Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln  
 245 250 255  
 Ser Gly Ala Glu Leu Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys  
 260 265 270  
 Lys Ala Ser Gly Tyr Ala Phe Thr Asn Tyr Trp Leu Gly Trp Val Lys  
 275 280 285  
 Gln Arg Pro Gly His Gly Leu Glu Trp Ile Gly Asp Leu Phe Pro Gly  
 290 295 300  
 Ser Gly Asn Thr His Tyr Asn Glu Arg Phe Arg Gly Lys Ala Thr Leu  
 305 310 315 320  
 Thr Ala Asp Lys Ser Ser Ser Thr Ala Phe Met Gln Leu Ser Ser Leu  
 325 330 335  
 Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg Leu Arg Asn Trp  
 340 345 350  
 Asp Glu Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser  
 355 360 365  
 Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
 370 375 380  
 Glu Leu Val Met Thr Gln Ser Pro Ser Tyr Leu Ala Ala Ser Pro Gly  
 385 390 395 400  
 Glu Thr Ile Thr Ile Asn Cys Arg Ala Ser Lys Ser Ile Ser Lys Tyr  
 405 410 415  
 Leu Ala Trp Tyr Gln Glu Lys Pro Gly Lys Thr Asn Lys Leu Leu Ile  
 420 425 430  
 Tyr Ser Gly Ser Thr Leu Gln Ser Gly Ile Pro Ser Arg Phe Ser Gly  
 435 440 445  
 Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro  
 450 455 460  
 Glu Asp Phe Ala Met Tyr Tyr Cys Gln Gln His Asn Glu Tyr Pro Tyr  
 465 470 475 480  
 Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys His His His His His  
 485 490 495

His

&lt;210&gt; 47

&lt;211&gt; 1494

&lt;212&gt; DNA

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; CD3 VHVL aL Ser x 3-1 VHVL

&lt;400&gt; 47

gatatcaaac tgcagcagtc aggggctgaa ctggcaagac ctggggcctc agtgaagatg	60
tcctgcaaga cttctggcta cacctttact aggtacacga tgcactgggt aaaacagagg	120
cctggacagg gtctggaatg gattggatac attaatccta gccgtgggta tactaattac	180
aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac	240
atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat	300
gatgatcatt actcccttga ctactggggc caaggcacca ctctcacagt ctctcagtc	360
gaagggtgaa gtggagggtc tgggtggaagt ggagggttcag gtggagtcga cgacattcag	420
ctgacccagt ctccagcaat catgtctgca tctccagggg agaaggtcac catgacctgc	480
agagccagtt caagtgtgaa ttacatgaac tgggtaccagc agaagtcagg cacctccccc	540
aaaagatgga tttatgacac atccaaagtg gcttctggag tcccttatcg cttcagtggc	600
agtgggtctg ggacctcata ctctctcaca atcagcagca tggaggctga agatgctgcc	660
acttattact gccaacagtg gagtagtaac ccgctcacgt tcgggtgctgg gaccaagctg	720
gagctgaaat ccggagggtg tggatccgag gtgcagctgc tcgagcagtc tggagctgag	780
ctggtgaaac ctggggcctc agtgaagata tcctgcaagg cttctggata cgccttact	840
aactactggc taggttgggt aaagcagagg cctggacatg gacttgagtg gattggagat	900
cttttccctg gaagtggtaa tactcactac aatgagaggt tcaggggcaa agccacactg	960
actgcagaca aatcctcgag cacagccttt atgcagctca gtagcctgac atctgaggac	1020
tctgctgtct atttctgtgc aagattgagg aactgggacg aggctatgga ctactggggc	1080
caagggacca cggtcaccgt ctctcaggt ggtggtggtt ctggcggcgg cggctccggt	1140
ggtggtggtt ctgagctcgt catgaccacg tctccatctt atcttgctgc atctcctgga	1200
gaaaccatta ctattaattg cagggcaagt aagagcatta gcaaataattt agcctggtat	1260
caagagaaac ctgggaaaac taataagctt cttatctact ctggatccac ttgcaatct	1320
ggaattccat caaggttcag tggcagtgga tctggtacag atttcactct caccatcagt	1380
agcctggagc ctgaagattt tgcaatgtat tactgtcaac agcataatga atatccgtac	1440
acgttcggag gggggaccaa gcttgagatc aaacatcatc accatcatca ttag	1494

53

&lt;210&gt; 48

&lt;211&gt; 497

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; CD3 VHVL aL Ser x 3-1 VHVL

&lt;400&gt; 48

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala  
 1 5 10 15

Ser Val Lys Met Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Arg Tyr  
 20 25 30

Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile  
 35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe  
 50 55 60

Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Thr Ala Tyr  
 65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys  
 85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Ser Leu Asp Tyr Trp Gly Gln Gly  
 100 105 110

Thr Thr Leu Thr Val Ser Ser Val Glu Gly Gly Ser Gly Gly Ser Gly  
 115 120 125

Gly Ser Gly Gly Ser Gly Gly Val Asp Asp Ile Gln Leu Thr Gln Ser  
 130 135 140

Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys  
 145 150 155 160

Arg Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser  
 165 170 175

Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser  
 180 185 190

Gly Val Pro Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser  
 195 200 205

54

Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys  
 210 215 220  
 Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu  
 225 230 235 240  
 Glu Leu Lys Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln  
 245 250 255  
 Ser Gly Ala Glu Leu Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys  
 260 265 270  
 Lys Ala Ser Gly Tyr Ala Phe Thr Asn Tyr Trp Leu Gly Trp Val Lys  
 275 280 285  
 Gln Arg Pro Gly His Gly Leu Glu Trp Ile Gly Asp Leu Phe Pro Gly  
 290 295 300  
 Ser Gly Asn Thr His Tyr Asn Glu Arg Phe Arg Gly Lys Ala Thr Leu  
 305 310 315 320  
 Thr Ala Asp Lys Ser Ser Ser Thr Ala Phe Met Gln Leu Ser Ser Leu  
 325 330 335  
 Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg Leu Arg Asn Trp  
 340 345 350  
 Asp Glu Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser  
 355 360 365  
 Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
 370 375 380  
 Glu Leu Val Met Thr Gln Ser Pro Ser Tyr Leu Ala Ala Ser Pro Gly  
 385 390 395 400  
 Glu Thr Ile Thr Ile Asn Cys Arg Ala Ser Lys Ser Ile Ser Lys Tyr  
 405 410 415  
 Leu Ala Trp Tyr Gln Glu Lys Pro Gly Lys Thr Asn Lys Leu Leu Ile  
 420 425 430  
 Tyr Ser Gly Ser Thr Leu Gln Ser Gly Ile Pro Ser Arg Phe Ser Gly  
 435 440 445  
 Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro  
 450 455 460  
 Glu Asp Phe Ala Met Tyr Tyr Cys Gln Gln His Asn Glu Tyr Pro Tyr  
 465 470 475 480

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys His His His His His  
 485 490 495

His

<210> 49

<211> 1521

<212> DNA

<213> artificial sequence

<220>

<223> CD3 VHVL aL x 3-5 VHVL

<400> 49

gatatcaaac tgcagcagtc aggggctgaa ctggcaagac ctggggcctc agtgaagatg	60
tcctgcaaga cttctggcta cacctttact aggtacacga tgcactgggt aaaacagagg	120
cctggacagg gtctggaatg gattggatac attaatccta gccgtgggta tactaattac	180
aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac	240
atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat	300
gatgatcatt actgccttga ctactggggc caaggcacca ctctcacagt ctctcagtc	360
gaagggtgaa gtggaggttc tgggtggaagt ggaggttcag gtggagtcga cgacattcag	420
ctgaccagtc ctccagcaat catgtctgca tctccagggg agaaggtcac catgacctgc	480
agagccagtt caagtgtgaa ttacatgaac tggtaggcagc agaagtcagg cacctcccc	540
aaaagatgga tttatgacac atccaaagtg gcttctggag tcccttatcg cttcagtggc	600
agtgggtctg ggacctcata ctctctcaca atcagcagca tggaggctga agatgctgcc	660
acttattact gccaacagtg gagtagtaac ccgctcacgt tcggtgctgg gaccaagctg	720
gagctgaaat ccggaggtgg tggatccgag gtgcagctgc tcgagcagtc tggagctgag	780
ctggtgaaggc ctgggacttc agtgaagctg tcctgcaagg cttctgggcta caccttcaca	840
agctatggtt taagctgggt gaagcagaga actggacagg gccttgagtg gattggagag	900
gtttatccta gaattggtaa tgcttactac aatgagaagt tcaagggcaa ggccacactg	960
actgcagaca aatcctccag cacagcgtcc atggagctcc gcagcctgac atctgaggac	1020
tctgcggctct atttctgtgc aagacgggga tcctacggtg gtaactacga ctgggtacttc	1080
gatgtctggg gccaaaggac cacggtcacc gtctcctcag gtgggtgggtg ttctggcggc	1140
ggcggctccg gtgggtgggtg ttctgagctc gtgatgaccc agactccact ctccctgcct	1200
gtcagtcttg gagatcaagc ctccatctct tgcagatcta gtcagagcct tgtacacagt	1260
aatggaaaca cctatttaca ttgggtacctg cagaagccag gccagtctcc aaagctcctg	1320
atctacaaag tttccaaccg attttctggg gtcccagaca gggttcagtg cagtggatca	1380

56

gggacagatt tcacactcaa gatcagcaga gtggaggctg aggatctggg agtttatttc 1440  
 tgctctcaaa gtacacatgt tccgtacacg ttcggagggg ggaccaagct tgagatcaaa 1500  
 catcatcacc atcatcatta g 1521

<210> 50

<211> 506

<212> PRT

<213> artificial sequence

<220>

<223> CD3 VHVL aL x 3-5 VHVL

<400> 50

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala  
 1 5 10 15

Ser Val Lys Met Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Arg Tyr  
 20 25 30

Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile  
 35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe  
 50 55 60

Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr  
 65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys  
 85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly  
 100 105 110

Thr Thr Leu Thr Val Ser Ser Val Glu Gly Gly Ser Gly Gly Ser Gly  
 115 120 125

Gly Ser Gly Gly Ser Gly Gly Val Asp Asp Ile Gln Leu Thr Gln Ser  
 130 135 140

Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys  
 145 150 155 160

Arg Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser  
 165 170 175

Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser  
 180 185 190



Gly Val Pro Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser  
 195 200 205  
 Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys  
 210 215 220  
 Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu  
 225 230 235 240  
 Glu Leu Lys Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln  
 245 250 255  
 Ser Gly Ala Glu Leu Val Arg Pro Gly Thr Ser Val Lys Leu Ser Cys  
 260 265 270  
 Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Gly Leu Ser Trp Val Lys  
 275 280 285  
 Gln Arg Thr Gly Gln Gly Leu Glu Trp Ile Gly Glu Val Tyr Pro Arg  
 290 295 300  
 Ile Gly Asn Ala Tyr Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu  
 305 310 315 320  
 Thr Ala Asp Lys Ser Ser Ser Thr Ala Ser Met Glu Leu Arg Ser Leu  
 325 330 335  
 Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg Arg Gly Ser Tyr  
 340 345 350  
 Gly Ser Asn Tyr Asp Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Thr  
 355 360 365  
 Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly  
 370 375 380  
 Gly Gly Gly Ser Glu Leu Val Met Thr Gln Thr Pro Leu Ser Leu Pro  
 385 390 395 400  
 Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser  
 405 410 415  
 Leu Val His Ser Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys  
 420 425 430  
 Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe  
 435 440 445  
 Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe  
 450 455 460

58

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe  
 465 470 475 480

Cys Ser Gln Ser Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys  
 485 490 495

Leu Glu Ile Lys His His His His His His  
 500 505

&lt;210&gt; 51

&lt;211&gt; 1521

&lt;212&gt; DNA

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; CD3 VHVL aL Ser x 3-5 VHVL

&lt;400&gt; 51

gatatcaaac tgcagcagtc aggggctgaa. ctggcaagac ctggggcctc agtgaagatg	60
tcctgcaaga cttctggcta cacctttact aggtacacga tgcactgggt aaaacagagg	120
cctggacagg gtctggaatg gattggatac attaatccta gccgtgggta tactaattac	180
aatcagaagt tcaaggacaa ggccaacattg actacagaca aatcctccag cacagcctac	240
atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat	300
gatgatcatt actcccttga ctactggggc caaggcacca ctctcacagt ctctcagtc	360
gaagggtggaa gtggagggtc tgggtggaagt ggagggttcag gtggagtcga cgacattcag	420
ctgacccagt ctccagcaat catgtctgca tctccagggg agaaggtcac catgacctgc	480
agagccagtt caagtgtaa ttacatgaac tgggtaccagc agaagtcagg cacctcccc	540
aaaagatgga tttatgacac atccaaagtg gcttctggag tcccttatcg cttcagtggc	600
agtgggtctg ggacctcata ctctctcaca atcagcagca tggaggctga agatgctgcc	660
acttattact gccaacagtg gagtagtaac ccgctcacgt tcggtgctgg gaccaagctg	720
gagctgaaat ccggagggtg tggatccgag gtgcagctgc tcgagcagtc tggagctgag	780
ctggtaaagg ctgggacttc agtgaagctg tcctgcaagg cttctggcta caccttcaca	840
agctatggtt taagctgggt gaagcagaga actggacagg gccttgagtg gattggagag	900
gtttatccta gaattggtaa tgcttactac aatgagaagt tcaagggcaa ggccacactg	960
actgcagaca aatcctccag cacagcgtcc atggagctcc gcagcctgac atctgaggac	1020
tctgcggtct atttctgtgc aagacgggga tcctacggta gtaactacga ctggtacttc	1080
gatgtctggg gccaagggac cacggtcacc gtctcctcag gtggtgggtg ttctggcggc	1140
ggcggctccg gtggtgggtg ttctgagctc gtgatgaccc agactccact ctccctgcct	1200
gtcagtcctg gagatcaagc ctccatctct tgcagatcta gtcagagcct tgtacacagt	1260

59

aatggaaaca cctatttaca ttggtacctg cagaagccag gccagtctcc aaagctcctg 1320  
 atctacaaag ttccaaccg attttctggg gtcccagaca ggttcagtgg cagtggatca 1380  
 gggacagatt tcacactcaa gatcagcaga gtggaggctg aggatctggg agtttatttc 1440  
 tgctctcaaa gtacacatgt tccgtacacg ttcggagggg ggaccaagct tgagatcaaa 1500  
 catcatcacc atcatcatta g 1521

&lt;210&gt; 52

&lt;211&gt; 506

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; CD3 VHVL aL Ser x 3-5 VHVL

&lt;400&gt; 52

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala  
 1 5 10 15

Ser Val Lys Met Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Arg Tyr  
 20 25 30

Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile  
 35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe  
 50 55 60

Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr  
 65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys  
 85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Ser Leu Asp Tyr Trp Gly Gln Gly  
 100 105 110

Thr Thr Leu Thr Val Ser Ser Val Glu Gly Gly Ser Gly Gly Ser Gly  
 115 120 125

Gly Ser Gly Gly Ser Gly Gly Val Asp Asp Ile Gln Leu Thr Gln Ser  
 130 135 140

Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys  
 145 150 155 160

Arg Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser

				165						170	60						175
Gly	Thr	Ser	Pro	Lys	Arg	Trp	Ile	Tyr	Asp	Thr	Ser	Lys	Val	Ala	Ser		
			180					185					190				
Gly	Val	Pro	Tyr	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Ser	Tyr	Ser		
		195					200					205					
Leu	Thr	Ile	Ser	Ser	Met	Glu	Ala	Glu	Asp	Ala	Ala	Thr	Tyr	Tyr	Cys		
	210					215					220						
Gln	Gln	Trp	Ser	Ser	Asn	Pro	Leu	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu		
225					230					235					240		
Glu	Leu	Lys	Ser	Gly	Gly	Gly	Gly	Ser	Glu	Val	Gln	Leu	Leu	Glu	Gln		
				245					250					255			
Ser	Gly	Ala	Glu	Leu	Val	Arg	Pro	Gly	Thr	Ser	Val	Lys	Leu	Ser	Cys		
			260					265					270				
Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Ser	Tyr	Gly	Leu	Ser	Trp	Val	Lys		
		275					280					285					
Gln	Arg	Thr	Gly	Gln	Gly	Leu	Glu	Trp	Ile	Gly	Glu	Val	Tyr	Pro	Arg		
	290					295					300						
Ile	Gly	Asn	Ala	Tyr	Tyr	Asn	Glu	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu		
305					310					315					320		
Thr	Ala	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Ser	Met	Glu	Leu	Arg	Ser	Leu		
				325					330					335			
Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys	Ala	Arg	Arg	Gly	Ser	Tyr		
			340					345					350				
Gly	Ser	Asn	Tyr	Asp	Trp	Tyr	Phe	Asp	Val	Trp	Gly	Gln	Gly	Thr	Thr		
		355					360					365					
Val	Thr	Val	Ser	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly		
	370					375					380						
Gly	Gly	Gly	Ser	Glu	Leu	Val	Met	Thr	Gln	Thr	Pro	Leu	Ser	Leu	Pro		
385					390					395					400		
Val	Ser	Leu	Gly	Asp	Gln	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser		
				405					410					415			
Leu	Val	His	Ser	Asn	Gly	Asn	Thr	Tyr	Leu	His	Trp	Tyr	Leu	Gln	Lys		
			420					425					430				
Pro	Gly	Gln	Ser	Pro	Lys	Leu	Leu	Ile	Tyr	Lys	Val	Ser	Asn	Arg	Phe		
		435					440					445					

61

Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe  
 450 455 460

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe  
 465 470 475 480

Cys Ser Gln Ser Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys  
 485 490 495

Leu Glu Ile Lys His His His His His His  
 500 505

<210> 53

<211> 1512

<212> DNA

<213> artificial sequence

<220>

<223> CD3 VHVL stL x 3-5 VHVL

<400> 53

gatatcaaac tgcagcagtc aggggctgaa ctggcaagac ctggggcctc agtgaagatg	60
tcctgcaaga cttctggcta cacctttact aggtacaaga tgcactgggt aaaacagagg	120
cctggacagg gtctggaatg gattggatac attaatccta gccgtgggta tactaattac	180
aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac	240
atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat	300
gatgatcatt actgccttga ctactggggc caaggcacca ctctcacagt ctctcaggt	360
gggtggtggt ctggcggcgg cggtccgggt ggtggtggt ctgacattca gctgaccag	420
tctccagcaa tcatgtctgc atctccaggg gagaagggtca ccatgacctg cagagccagt	480
tcaagtgtaa gttacatgaa ctggtaccag cagaagtcag gcacctccc caaaagatgg	540
atttatgaca catcaaagt ggcttctgga gtcccttacc gcttcagtgg cagtgggtct	600
gggacctcat actctctcac aatcagcagc atggaggctg aagatgctgc cacttattac	660
tgccaacagt ggagtagtaa cccgctcacg ttcgggtgctg ggaccaagct ggagctgaaa	720
tccggagggt gtggatccga ggtgcagctg ctcgagcagt ctggagctga gctggtaagg	780
cctgggactt cagtgaagct gtcctgcaag gcttctggct acaccttcac aagctatggt	840
ttaagctggg tgaagcagag aactggacag ggccttgagt ggattggaga ggtttatcct	900
agaattggtg atgcttacta caatgagaag ttcaagggca aggccacact gactgcagac	960
aaatcctcca gcacagcgtc catggagctc cgcagcctga catctgagga ctctgcggtc	1020
tatttctgtg caagacgggg atcctacgggt agtaactacg actgggtactt cgatgtctgg	1080

62

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ggccaagga ccacggcac cgtctcctca ggtggtggtg gttctggcgg cggcggctcc 1140
ggtggtggtg gttctgagct cgtgatgacc cagactccac tctccctgcc tgtcagtctt 1200
ggagatcaag cctccatctc ttgcagatct agtcagagcc ttgtacacag taatggaaac 1260
acctatttac attggtacct gcagaagcca ggccagtctc caaagctcct gatctacaaa 1320
gtttccaacc gattttctgg ggtcccagac aggttcagtg gcagtggatc agggacagat 1380
ttcactca agatcagcag agtggaggct gaggatctgg gagtttattt ctgctctcaa 1440
agtacacatg ttccgtacac gttcggaggg gggaccaagc ttgagatcaa acatcatcac 1500
catcatcatt ag 1512

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&lt;210&gt; 54

&lt;211&gt; 503

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; CD3 VHVL stL x 3-5 VHVL

&lt;400&gt; 54

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala  
1 5 10 15

Ser Val Lys Met Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Arg Tyr  
20 25 30

Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile  
35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe  
50 55 60

Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr  
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys  
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly  
100 105 110

Thr Thr Leu Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly  
115 120 125

Ser Gly Gly Gly Gly Ser Asp Ile Gln Leu Thr Gln Ser Pro Ala Ile  
130 135 140

Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser  
 145 150 155 160

Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser  
 165 170 175

Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser Gly Val Pro  
 180 185 190

Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile  
 195 200 205

Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp  
 210 215 220

Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys  
 225 230 235 240

Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln Ser Gly Ala  
 245 250 255

Glu Leu Val Arg Pro Gly Thr Ser Val Lys Leu Ser Cys Lys Ala Ser  
 260 265 270

Gly Tyr Thr Phe Thr Ser Tyr Gly Leu Ser Trp Val Lys Gln Arg Thr  
 275 280 285

Gly Gln Gly Leu Glu Trp Ile Gly Glu Val Tyr Pro Arg Ile Gly Asn  
 290 295 300

Ala Tyr Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp  
 305 310 315 320

Lys Ser Ser Ser Thr Ala Ser Met Glu Leu Arg Ser Leu Thr Ser Glu  
 325 330 335

Asp Ser Ala Val Tyr Phe Cys Ala Arg Arg Gly Ser Tyr Gly Ser Asn  
 340 345 350

Tyr Asp Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val  
 355 360 365

Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly  
 370 375 380

Ser Glu Leu Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu  
 385 390 395 400

Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His  
 405 410 415

Ser Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln

64

420                      425                      430

Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val  
           435                                      440                                      445

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys  
           450                                      455                                      460

Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln  
           465                                      470                                      475                                      480

Ser Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile  
                                   485                                      490                                      495

Lys His His His His His His  
                                   500

&lt;210&gt; 55

&lt;211&gt; 1512

&lt;212&gt; DNA

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; CD3 VHVL aL x 4-1 VHVL

&lt;400&gt; 55

gatatcaaac tgcagcagtc aggggctgaa ctggcaagac ctggggcctc agtgaagatg	60
tcctgcaaga cttctggcta cacctttact aggtacacga tgcactgggt aaaacagagg	120
cctggacagg gtctggaatg gattggatac attaatccta gccgtggtta tactaattac	180
aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac	240
atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat	300
gatgatcatt actgccttga ctactggggc caaggcacca ctctcacagt ctctcagtc	360
gaagggtgaa gtggaggttc tgggtggaagt ggagggttcag gtggagtcga cgacattcag	420
ctgaccagtc ctccagcaat catgtctgca tctccagggg agaaggtcac catgacctgc	480
agagccagtt caagtgtgaa ttacatgaac tgggtaccagc agaagtcagg cacctcccc	540
aaaagatgga tttatgacac atccaaagtg gcttctggag tcccttatcg cttcagtggc	600
agtgggtctg ggacctcata ctctctcaca atcagcagca tggaggctga agatgtgcc	660
acttattact gccaacagtg gagtagtaac ccgctcacgt tcggtgctgg gaccaagctg	720
gagctgaaat ccggaggttg tggatccgag gtgcagctgc tcgagcagtc tggagctgag	780
ctggtgaaggc ctgggacttc agtgaagata tcctgcaagg cttctggata cgccttcact	840
aactactggc taggttgggt taagcagagg cctggacatg gacttgaatg ggttggagat	900
attttcctg gaagtggtaa tgctcactac aatgagaagt tcaagggcaa agccacactg	960



65

actgcagaca agtcctcgta cacagcctat atgcagctca gtagcctgac atctgaggac 1020  
 tctgctgtct atttctgtgc aagattgcgg aactgggacg aggctatgga ctactggggc 1080  
 caagggacca cggtcaccgt ctcctcaggt ggtggtggtt ctggcggcgg cggctccggt 1140  
 ggtggtggtt ctgagctcgt gatgacacag tctccatcct ccctgagtgt gtcagcagga 1200  
 gagaaggta ctatgagctg caagtccagt cagagtctgt taaacagtgg aaatcaaaag 1260  
 aactacttgg cctggtacca gcagaaacca gggcagcctc ctaaactggt gatctacggg 1320  
 gcattccacta gggaatctgg ggtccctgat cgcttcacag gcagtggatc tggaacagat 1380  
 ttactctca ccatcagcag tgtgcaggct gaagacctgg cagtttatta ctgtcagaat 1440  
 gattatagtt atccgtacac gttcggaggg gggaccaagc ttgagatcaa acatcatcac 1500  
 catcatcatt ag 1512

&lt;210&gt; 56

&lt;211&gt; 503

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; CD3 VHVL aL x 4-1 VHVL

&lt;400&gt; 56

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala  
 1 5 10 15

Ser Val Lys Met Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Arg Tyr  
 20 25 30

Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile  
 35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe  
 50 55 60

Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr  
 65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys  
 85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly  
 100 105 110

Thr Thr Leu Thr Val Ser Ser Val Glu Gly Gly Ser Gly Gly Ser Gly  
 115 120 125

66

Gly Ser Gly Gly Ser Gly Gly Val Asp Asp Ile Gln Leu Thr Gln Ser  
130 135 140

Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys  
145 150 155 160

Arg Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser  
165 170 175

Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser  
180 185 190

Gly Val Pro Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser  
195 200 205

Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys  
210 215 220

Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu  
225 230 235 240

Glu Leu Lys Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln  
245 250 255

Ser Gly Ala Glu Leu Val Arg Pro Gly Thr Ser Val Lys Ile Ser Cys  
260 265 270

Lys Ala Ser Gly Tyr Ala Phe Thr Asn Tyr Trp Leu Gly Trp Val Lys  
275 280 285

Gln Arg Pro Gly His Gly Leu Glu Trp Val Gly Asp Ile Phe Pro Gly  
290 295 300

Ser Gly Asn Ala His Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu  
305 310 315 320

Thr Ala Asp Lys Ser Ser Tyr Thr Ala Tyr Met Gln Leu Ser Ser Leu  
325 330 335

Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg Leu Arg Asn Trp  
340 345 350

Asp Glu Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser  
355 360 365

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
370 375 380

Glu Leu Val Met Thr Gln Ser Pro Ser Ser Leu Ser Val Ser Ala Gly  
385 390 395 400

Glu Lys Val Thr Met Ser Cys Lys Ser Ser<sup>67</sup> Gln Ser Leu Leu Asn Ser  
 405 410 415

Gly Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln  
 420 425 430

Pro Pro Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg Glu Ser Gly Val  
 435 440 445

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr  
 450 455 460

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn  
 465 470 475 480

Asp Tyr Ser Tyr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile  
 485 490 495

Lys His His His His His His  
 500

<210> 57

<211> 1512

<212> DNA

<213> artificial sequence

<220>

<223> CD3 VHVL aL Ser x 4-1 VHVL

<400> 57  
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 tcctgcaaga cttctggcta cacctttact aggtacacga tgcactgggt aaaacagagg 120  
 cctggacagg gtctggaatg gattggatac attaatecta gccgtgggta tactaattac 180  
 aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac 240  
 atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat 300  
 gatgatcatt actcccttga ctactggggc caaggcacca ctctcacagt ctctcagtc 360  
 gaagggtgaa gtggaggttc tgggtggaagt ggaggttcag gtggagtcga cgacattcag 420  
 ctgaccagtc ctccagcaat catgtctgca tctccagggg agaaggtcac catgacctgc 480  
 agagccagtt caagtgtgaa ttacatgaac tggtagcagc agaagtcagg cacctccccc 540  
 aaaagatgga tttatgacac atccaaagtg gcttctggag tcccttatcg cttcagtggtc 600  
 agtgggtctg ggacctcata ctctctcaca atcagcagca tggaggctga agatgctgcc 660  
 acttattact gccaacagtg gagtagtaac ccgctcacgt tcggtgctgg gaccaagctg 720  
 gagctgaaat ccggaggttg tggatccgag gtgcagctgc tcgagcagtc tggagctgag 780

68

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ctggttaaggc ctgggacttc agtgaagata tcctgcaagg cttctggata cgccttcact 840
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attttccctg gaagtggtaa tgctcactac aatgagaagt tcaagggcaa agccacactg 960
actgcagaca agtcctcgta cacagcctat atgcagctca gtagcctgac atctgaggac 1020
tctgtgtctt atttctgtgc aagattgcgg aactgggacg aggctatgga ctactggggc 1080
caagggacca cggtcaccgt ctcttcaggt ggtggtgggt ctggcggcgg cggctccggt 1140
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gagaagggtca ctatgagctg caagtccagt cagagtctgt taaacagtgg aaatcaaaag 1260
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gcattccacta gggaatctgg ggtccctgat cgcttcacag gcagtggatc tggaacagat 1380
ttcactctca ccatcagcag tgtgcaggct gaagacctgg cagtttatta ctgtcagaat 1440
gattatagtt atccgtacac gttcggaggg gggaccaagc ttgagatcaa acatcatcac 1500
catcatcatt ag 1512

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&lt;210&gt; 58

&lt;211&gt; 503

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; CD3 VHVL aL Ser x 4-1 VHVL

&lt;400&gt; 58

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala  
1 5 10 15

Ser Val Lys Met Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Arg Tyr  
20 25 30

Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile  
35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe  
50 55 60

Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr  
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys  
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Ser Leu Asp Tyr Trp Gly Gln Gly  
100 105 110

Thr Thr Leu Thr Val Ser Ser Val Glu Gly Gly Ser Gly Gly Ser Gly  
 115 120 125  
 Gly Ser Gly Gly Ser Gly Gly Val Asp Asp Ile Gln Leu Thr Gln Ser  
 130 135 140  
 Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys  
 145 150 155 160  
 Arg Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser  
 165 170 175  
 Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser  
 180 185 190  
 Gly Val Pro Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser  
 195 200 205  
 Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys  
 210 215 220  
 Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu  
 225 230 235 240  
 Glu Leu Lys Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln  
 245 250 255  
 Ser Gly Ala Glu Leu Val Arg Pro Gly Thr Ser Val Lys Ile Ser Cys  
 260 265 270  
 Lys Ala Ser Gly Tyr Ala Phe Thr Asn Tyr Trp Leu Gly Trp Val Lys  
 275 280 285  
 Gln Arg Pro Gly His Gly Leu Glu Trp Val Gly Asp Ile Phe Pro Gly  
 290 295 300  
 Ser Gly Asn Ala His Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu  
 305 310 315 320  
 Thr Ala Asp Lys Ser Ser Tyr Thr Ala Tyr Met Gln Leu Ser Ser Leu  
 325 330 335  
 Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg Leu Arg Asn Trp  
 340 345 350  
 Asp Glu Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser  
 355 360 365  
 Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
 370 375 380

70

Glu Leu Val Met Thr Gln Ser Pro Ser Ser Leu Ser Val Ser Ala Gly  
385 390 395 400

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser  
405 410 415

Gly Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln  
420 425 430

Pro Pro Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg Glu Ser Gly Val  
435 440 445

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr  
450 455 460

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn  
465 470 475 480

Asp Tyr Ser Tyr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile  
485 490 495

Lys His His His His His  
500

<210> 59

<211> 1503

<212> DNA

<213> artificial sequence

<220>

<223> CD3 VHVL stL x 4-1 VHVL

<400> 59

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cctggacagg gtctggaatg gattggatac attaatccta gccgtgggta tactaattac	180
aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac	240
atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat	300
gatgatcatt actgccttga ctactggggc caaggcacca ctctcacagt ctcctcaggt	360
ggtggtggtt ctggcggcgg cggctccggt ggtggtggtt ctgacattca gctgaccag	420
tctccagcaa tcatgtctgc atctccaggg gagaaggta ccatgacctg cagagccagt	480
tcaagtgtaa gttacatgaa ctggtaccag cagaagtcag gcacctcccc caaagatgg	540
atttatgaca catccaaagt ggcttctgga gtcccttata gcttcagtgg cagtgggtct	600
gggacctcat actctctcac aatcagcagc atggaggctg aagatgctgc cacttattac	660

71

tgccaacagt ggagtagtaa cccgctcacg ttcggtgctg ggaccaagct ggagctgaaa 720  
 tccggagggtg gtggatccga ggtgcagctg ctcgagcagt ctggagctga gctggtaagg 780  
 cctgggactt cagtgaagat atcctgcaag gcttctggat acgccttcac taactactgg 840  
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 ggaagtggta atgctcacta caatgagaag ttcaagggca aagccacact gactgcagac 960  
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 accatcagca gtgtgcaggc tgaagacctg gcagtttatt actgtcagaa tgattatagt 1440  
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 tag 1503

&lt;210&gt; 60

&lt;211&gt; 500

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; CD3 VHVL stL x 4-1 VHVL

&lt;400&gt; 60

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala  
 1 5 10 15

Ser Val Lys Met Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Arg Tyr  
 20 25 30

Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile  
 35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe  
 50 55 60

Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr  
 65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys

85 90 72 95  
 Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly  
 100 105 110  
 Thr Thr Leu Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly  
 115 120 125  
 Ser Gly Gly Gly Gly Ser Asp Ile Gln Leu Thr Gln Ser Pro Ala Ile  
 130 135 140  
 Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser  
 145 150 155 160  
 Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser  
 165 170 175  
 Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser Gly Val Pro  
 180 185 190  
 Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile  
 195 200 205  
 Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp  
 210 215 220  
 Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys  
 225 230 235 240  
 Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Gln Ser Gly Ala  
 245 250 255  
 Glu Leu Val Arg Pro Gly Thr Ser Val Lys Ile Ser Cys Lys Ala Ser  
 260 265 270  
 Gly Tyr Ala Phe Thr Asn Tyr Trp Leu Gly Trp Val Lys Gln Arg Pro  
 275 280 285  
 Gly His Gly Leu Glu Trp Val Gly Asp Ile Phe Pro Gly Ser Gly Asn  
 290 295 300  
 Ala His Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp  
 305 310 315 320  
 Lys Ser Ser Tyr Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu  
 325 330 335  
 Asp Ser Ala Val Tyr Phe Cys Ala Arg Leu Arg Asn Trp Asp Glu Ala  
 340 345 350  
 Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly  
 355 360 365



Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Leu Val  
 370 375 380

Met Thr Gln Ser Pro Ser Ser Leu Ser Val Ser Ala Gly Glu Lys Val  
 385 390 395 400

Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser Gly Asn Gln  
 405 410 415

Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys  
 420 425 430

Leu Leu Ile Tyr Gly Ala Ser Thr Arg Glu Ser Gly Val Pro Asp Arg  
 435 440 445

Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser  
 450 455 460

Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn Asp Tyr Ser  
 465 470 475 480

Tyr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys His His  
 485 490 495

His His His His  
 500

<210> 61

<211> 10

<212> PRT

<213> artificial sequence

<220>

<223> CDRH3 M1 mutant

<400> 61

His Tyr Asp Asp His Tyr Cys Leu Asp Tyr  
 1 5 10

<210> 62

<211> 10

<212> PRT

<213> artificial sequence

<220>

<223> CDRH3 M4 mutant

<400> 62

Tyr Ser Asp Asp His Tyr Cys Leu Asp Tyr  
1 5 10

<210> 63

<211> 10

<212> PRT

<213> artificial sequence

<220>

<223> CDRH3 M7 mutant

<400> 63

Tyr Tyr Asp Ala His Tyr Cys Leu Asp Tyr  
1 5 10

<210> 64

<211> 10

<212> PRT

<213> artificial sequence

<220>

<223> CDRH3 M9 mutant

<400> 64

Tyr Tyr Asp Asp Gln Tyr Cys Leu Asp Tyr  
1 5 10

<210> 65

<211> 10

<212> PRT

<213> artificial sequence

<220>

<223> CDRH3 M10 mutant

<400> 65

Tyr Tyr Asp Asp Pro Tyr Cys Leu Asp Tyr  
1 5 10

75

&lt;210&gt; 66

&lt;211&gt; 10

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; CDRH3 M11 mutant

&lt;400&gt; 66

Tyr Phe Asn Asp His Tyr Cys Leu Asp Tyr  
1 5 10

&lt;210&gt; 67

&lt;211&gt; 10

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; CDRH3 M13 mutant

&lt;400&gt; 67

Tyr Tyr Asn Asp Gln Tyr Cys Leu Asp Tyr  
1 5 10

&lt;210&gt; 68

&lt;211&gt; 10

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; CDRH3 M20 mutant

&lt;400&gt; 68

Tyr His Asp Asp Pro Tyr Cys Leu Asp Tyr  
1 5 10

&lt;210&gt; 69

&lt;211&gt; 10

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; CDRH3 M76 mutant

&lt;400&gt; 69

Tyr Tyr Asp Asp Asn Tyr Cys Leu Asp Tyr  
 1 5 10

&lt;210&gt; 70

&lt;211&gt; 18

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; original linker

&lt;400&gt; 70

Val Glu Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly  
 1 5 10 15

Val Asp

&lt;210&gt; 71

&lt;211&gt; 357

&lt;212&gt; DNA

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; anti-CD3 VH

&lt;400&gt; 71

gatatcaaac tgcagcagtc aggggctgaa ctggcaagac ctggggcctc agtgaagatg	60
tcctgcaaga cttctggcta cacctttact aggtacacga tgcactgggt aaaacagagg	120
cctggacagg gtctggaatg gattggatac attaatccta gccgtgggta tactaattac	180
aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac	240
atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat	300
gatgatcatt actgccttga ctactggggc caaggcacca ctctcacagt ctctctca	357

&lt;210&gt; 72

&lt;211&gt; 119

&lt;212&gt; PRT

77

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; anti-CD3VH

&lt;400&gt; 72

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala  
 1 5 10 15

Ser Val Lys Met Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Arg Tyr  
 20 25 30

Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile  
 35 40 45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe  
 50 55 60

Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr  
 65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys  
 85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly  
 100 105 110

Thr Thr Leu Thr Val Ser Ser  
 115

&lt;210&gt; 73

&lt;211&gt; 318

&lt;212&gt; DNA

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; anti-CD3 VL

&lt;400&gt; 73

gacattcagc tgaccagtc tccagcaatc atgtctgcat ctccagggga gaaggtcacc	60
atgacctgca gagccagttc aagtgttaagt tacatgaact ggtaccagca gaagtcaggc	120
acctccccca aaagatggat ttatgacaca tccaaagtgg cttctggagt cccttatcgc	180
ttcagtggca gtgggtctgg gacctcatc tctctcaca tcagcagcat ggaggctgaa	240
gatgctgccca cttattactg ccaacagtgg agtagtaacc cgctcacgtt cggtgctggg	300
accaagctgg agctgaaa	318

78

&lt;210&gt; 74

&lt;211&gt; 106

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; anti-CD3 VL

&lt;400&gt; 74

Asp Ile Gln Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly  
 1 5 10 15

Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Met  
 20 25 30

Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr  
 35 40 45

Asp Thr Ser Lys Val Ala Ser Gly Val Pro Tyr Arg Phe Ser Gly Ser  
 50 55 60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu  
 65 70 75 80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Leu Thr  
 85 90 95

Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys  
 100 105

&lt;210&gt; 75

&lt;211&gt; 10

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; vH CDR1 anti-CD3

&lt;400&gt; 75

Gly Tyr Thr Phe Thr Arg Tyr Thr Met His  
 1 5 10

&lt;210&gt; 76

&lt;211&gt; 357

&lt;212&gt; . DNA

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; vH anti-CD3 cys-&gt;ser

&lt;400&gt; 76

gatatcaaac tgcagcagtc aggggctgaa ctggcaagac ctggggcctc agtgaagatg 60  
tcctgcaaga cttctggcta cacctttact aggtacacga tgcactgggt aaaacagagg 120  
cctggacagg gtctggaatg gattggatac attaatccta gccgtgggtta tactaattac 180  
aatcagaagt tcaaggacaa ggccacattg actacagaca aatcctccag cacagcctac 240  
atgcaactga gcagcctgac atctgaggac tctgcagtct attactgtgc aagatattat 300  
gatgatcatt actcccttga ctactggggc caaggcacca ctctcacagt ctctctca 357

&lt;210&gt; 77

&lt;211&gt; 119

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; vH anti-CD3 cys-&gt;ser

&lt;400&gt; 77

Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala  
1 5 10 15  
Ser Val Lys Met Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Arg Tyr  
20 25 30  
Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile  
35 40 45  
Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe  
50 55 60  
Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr  
65 70 75 80  
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys  
85 90 95  
Ala Arg Tyr Tyr Asp Asp His Tyr Ser Leu Asp Tyr Trp Gly Gln Gly  
100 105 110  
Thr Thr Leu Thr Val Ser Ser

80

115

&lt;210&gt; 78

&lt;211&gt; 10

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; VH CDR3 anti-CD3 cys-&gt;ser

&lt;400&gt; 78

Tyr	Tyr	Asp	Asp	His	Tyr	Ser	Leu	Asp	Tyr
1				5					10

&lt;210&gt; 79

&lt;211&gt; 360

&lt;212&gt; DNA

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; EpCAM 3-1 VH

&lt;400&gt; 79

gaggtgcagc	tgctcgagca	gtctggagct	gagctggtga	aacctggggc	ctcagtgaag	60
atatcctgca	aggcttctgg	atacgccttc	actaactact	ggctagggtg	ggtaaagcag	120
aggcctggac	atggacttga	gtggattgga	gatcttttcc	ctggaagtgg	taatactcac	180
tacaatgaga	ggttcagggg	caaagccaca	ctgactgcag	acaaatcctc	gagcacagcc	240
tttatgcagc	tcagtagcct	gacatctgag	gactctgctg	tctatttctg	tgcaagattg	300
aggaactggg	acgaggctat	ggactactgg	ggccaaggga	ccacgggtcac	cgtctcctca	360

&lt;210&gt; 80

&lt;211&gt; 120

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; EpCAM 3-1 VH

&lt;400&gt; 80

Glu	Val	Gln	Leu	Leu	Glu	Gln	Ser	Gly	Ala	Glu	Leu	Val	Lys	Pro	Gly
1				5					10					15	



81

Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Thr Asn  
 20 25 30

Tyr Trp Leu Gly Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp  
 35 40 45

Ile Gly Asp Leu Phe Pro Gly Ser Gly Asn Thr His Tyr Asn Glu Arg  
 50 55 60

Phe Arg Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala  
 65 70 75 80

Phe Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe  
 85 90 95

Cys Ala Arg Leu Arg Asn Trp Asp Glu Ala Met Asp Tyr Trp Gly Gln  
 100 105 110

Gly Thr Thr Val Thr Val Ser Ser  
 115 120

&lt;210&gt; 81

&lt;211&gt; 321

&lt;212&gt; DNA

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; EpCAM 3-1 VL

<400> 81  
 gagctcgtca tgaccagtc tccatcttat ctgctgcat ctctggaga aaccattact 60  
 attaattgca gggcaagtaa gagcattagc aaatatttag cctggtatca agagaaacct 120  
 gggaaaacta ataagcttct tatctactct ggatccactt tgcaatctgg aattccatca 180  
 aggttcagtg gcagtggatc tggtagatc ttactctca ccatcagtag cctggagcct 240  
 gaagattttg caatgtatta ctgtcaacag cataatgaat atccgtacac gttcggaggg 300  
 gggaccaagc ttgagatcaa a 321

&lt;210&gt; 82

&lt;211&gt; 107

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

82

&lt;223&gt; EpCAM 3-1 VL

&lt;400&gt; 82

Glu Leu Val Met Thr Gln Ser Pro Ser Tyr Leu Ala Ala Ser Pro Gly  
 1 5 10 15

Glu Thr Ile Thr Ile Asn Cys Arg Ala Ser Lys Ser Ile Ser Lys Tyr  
 20 25 30

Leu Ala Trp Tyr Gln Glu Lys Pro Gly Lys Thr Asn Lys Leu Leu Ile  
 35 40 45

Tyr Ser Gly Ser Thr Leu Gln Ser Gly Ile Pro Ser Arg Phe Ser Gly  
 50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro  
 65 70 75 80

Glu Asp Phe Ala Met Tyr Tyr Cys Gln Gln His Asn Glu Tyr Pro Tyr  
 85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
 100 105

&lt;210&gt; 83

&lt;211&gt; 372

&lt;212&gt; DNA

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; EpCAM 3-5 VH

&lt;400&gt; 83

gaggtgcagc tgctcgagca gtctggagct gagctggtaa ggcctgggac ttcagtgaag 60  
 ctgtcctgca aggttctctg ctacacctc acaagctatg gtttaagctg ggtgaagcag 120  
 agaactggac agggccttga gtggattgga gaggtttatc ctagaattgg taatgcttac 180  
 tacaatgaga agttcaaggg caaggccaca ctgactgcag acaaatcctc cagcacagcg 240  
 tccatggagc tccgcagcct gacatctgag gactctgcgg tctatttctg tgcaagacgg 300  
 ggatcctacg gtagtaacta cgactggtac ttcgatgtct ggggccaagg gaccacggtc 360  
 accgtctcct ca 372

&lt;210&gt; 84

&lt;211&gt; 124

&lt;212&gt; PRT

83

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; EpCAM 3-5 VH

&lt;400&gt; 84

Glu Val Gln Leu Leu Glu Gln Ser Gly Ala Glu Leu Val Arg Pro Gly  
 1 5 10 15

Thr Ser val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser  
 20 25 30

Tyr Gly Leu Ser Trp Val Lys Gln Arg Thr Gly Gln Gly Leu Glu Trp  
 35 40 45

Ile Gly Glu Val Tyr Pro Arg Ile Gly Asn Ala Tyr Tyr Asn Glu Lys  
 50 55 60

Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala  
 65 70 75 80

Ser Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe  
 85 90 95

Cys Ala Arg Arg Gly Ser Tyr Gly Ser Asn Tyr Asp Trp Tyr Phe Asp  
 100 105 110

Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser  
 115 120

&lt;210&gt; 85

&lt;211&gt; 336

&lt;212&gt; DNA

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; EpCAM 3-5 VL

&lt;400&gt; 85

gagctcgtga tgaccagac tccactctcc ctgcctgtca gtcttggaga tcaagcctcc 60

atctcttgca gatctagtca gaggcttgta cacagtaatg gaaacaccta tttacattgg 120

tacctgcaga agccaggcca gtctccaaag ctctgatct acaaagtttc caaccgattt 180

tctgggggtcc cagacagggtt cagtggcagt ggatcaggga cagatttcac actcaagatc 240

agcagagtgg aggctgagga tctgggagtt tatttctgct ctcaaagtac acatgtttccg 300

tacacgttcg gaggggggac caagcttgag atcaaa 336

84

&lt;210&gt; 86

&lt;211&gt; 112

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; EpCAM 3-5 VL

&lt;400&gt; 86

Glu Leu Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly  
 1 5 10 15

Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser  
 20 25 30

Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser  
 35 40 45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro  
 50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile  
 65 70 75 80

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser  
 85 90 95

Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
 100 105 110

&lt;210&gt; 87

&lt;211&gt; 360

&lt;212&gt; DNA

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; EpCAM 4-1 VH

&lt;400&gt; 87

gaggtgcagc tgctcgagca gtctggagct gagctggtaa ggcctgggac ttcagtgaag	60
atatcctgca aggcttctgg atacgccttc actaactact ggctaggttg ggtaagcag	120
aggcctggac atggacttga atgggttga gatattttcc ctggaagtgg taatgctcac	180
tacaatgaga agttcaaggg caaagccaca ctgactgcag acaagtcctc gtacacagcc	240
tatatgcagc tcagtagcct gacatctgag gactctgctg tctatttctg tgcaagattg	300

85

cggaactggg acgaggctat ggactactgg ggccaaggga ccacgggtcac cgtctcctca 360

<210> 88

<211> 120

<212> PRT

<213> artificial sequence

<220>

<223> EpCAM 4-1 VH

<400> 88

Glu Val Gln Leu Leu Glu Gln Ser Gly Ala Glu Leu Val Arg Pro Gly  
1 5 10 15

Thr Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Thr Asn  
20 25 30

Tyr Trp Leu Gly Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp  
35 40 45

Val Gly Asp Ile Phe Pro Gly Ser Gly Asn Ala His Tyr Asn Glu Lys  
50 55 60

Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Tyr Thr Ala  
65 70 75 80

Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe  
85 90 95

Cys Ala Arg Leu Arg Asn Trp Asp Glu Ala Met Asp Tyr Trp Gly Gln  
100 105 110

Gly Thr Thr Val Thr Val Ser Ser  
115 120

<210> 89

<211> 339

<212> DNA

<213> artificial sequence

<220>

<223> EpCAM 4-1 VL

<400> 89

gagctcgtga tgacacagtc tccatcctcc ctgagtgtgt cagcaggaga gaaggtcact 60

86

atgagctgca agtccagtca gagtctgtta aacagtggaa atcaaaagaa ctacttggcc	120
tggtaccagc agaaaccagg gcagcctcct aaactgttga tctacggggc atccactagg	180
gaatctgggg tccctgatcg cttcacaggc agtggatctg gaacagattt cactctcacc	240
atcagcagtg tgcaggctga agacctggca gtttattact gtcagaatga ttatagttat	300
ccgtacacgt tcggaggggg gaccaagctt gagatcaaa	339

&lt;210&gt; 90

&lt;211&gt; 113

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; EpCAM 4-1 VL

&lt;400&gt; 90

Glu	Leu	Val	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Val	Ser	Ala	Gly
1				5					10					15	

Glu	Lys	Val	Thr	Met	Ser	Cys	Lys	Ser	Ser	Gln	Ser	Leu	Leu	Asn	Ser
			20					25					30		

Gly	Asn	Gln	Lys	Asn	Tyr	Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln
	35						40					45			

Pro	Pro	Lys	Leu	Leu	Ile	Tyr	Gly	Ala	Ser	Thr	Arg	Glu	Ser	Gly	Val
	50					55					60				

Pro	Asp	Arg	Phe	Thr	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr
65					70					75				80	

Ile	Ser	Ser	Val	Gln	Ala	Glu	Asp	Leu	Ala	Val	Tyr	Tyr	Cys	Gln	Asn
				85					90					95	

Asp	Tyr	Ser	Tyr	Pro	Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile
			100					105					110		

Lys

&lt;210&gt; 91

&lt;211&gt; 372

&lt;212&gt; DNA

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; EpCAM 4-7 VH

&lt;400&gt; 91

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gagggtgcagc tgctcgagca gtctggagct gagctggcga ggcctggggc ttcagtgaag      60
ctgtcctgca aggccttctgg ctacaccttc acaaactatg gtttaagctg ggtgaagcag      120
aggcctggac aggtccttga gtggattgga gaggtttatc ctagaattgg taatgcttac      180
tacaatgaga agttcaaggg caaggccaca ctgactgcag acaaatcctc cagcacagcg      240
tccatggagc tccgcagcct gacctctgag gactctgcgg tctatttctg tgcaagacgg      300
ggatcctacg atactaacta cgactggtac ttcgatgtct ggggccaagg gaccacggtc      360
accgtctcct ca                                                              372

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&lt;210&gt; 92

&lt;211&gt; 124

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; EpCAM 4-7 VH

&lt;400&gt; 92

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Glu Val Gln Leu Leu Glu Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly
1           5           10           15

Ala Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn
20          25          30

Tyr Gly Leu Ser Trp Val Lys Gln Arg Pro Gly Gln Val Leu Glu Trp
35          40          45

Ile Gly Glu Val Tyr Pro Arg Ile Gly Asn Ala Tyr Tyr Asn Glu Lys
50          55          60

Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala
65          70          75          80

Ser Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe
85          90          95

Cys Ala Arg Arg Gly Ser Tyr Asp Thr Asn Tyr Asp Trp Tyr Phe Asp
100         105         110

Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115         120

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&lt;210&gt; 93

&lt;211&gt; 336

&lt;212&gt; DNA

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; EpCAM 4-7 VL

&lt;400&gt; 93

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tacctgcaga agccaggcca gtctccaaag ctctgatct acaaagtttc caaccgattt	180
tctgggggtcc cagacaggtt cagtggcagt ggatcaggga cagatttcac actcaagatc	240
agcagagtgg aggctgagga tctgggagtt tttttctgct ctcaaagtac acatgttccg	300
tacacgttcg gaggggggac caagcttgag atcaaa	336

&lt;210&gt; 94

&lt;211&gt; 112

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; EpCAM 4-7 VL

&lt;400&gt; 94

Glu Leu Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly	1	5	10	15
Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser	20	25	30	
Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser	35	40	45	
Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro	50	55	60	
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile	65	70	75	80
Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser	85	90	95	
Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys	100	105	110	



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 <212> DNA  
 <213> artificial sequence

<220>

<223> EpCAM 5-10 VH

<400> 95  
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 aggcctggac atggacttga gtggattgga gatattttcc ctggaagtgg taatatccac 180  
 tacaatgaga agttcaaggg caaagccaca ctgactgcag acaaattctc gagcacagcc 240  
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<210> 96  
 <211> 120  
 <212> PRT  
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<220>

<223> EpCAM 5-10 VH

<400> 96

Glu Val Gln Leu Leu Glu Gln Ser Gly Ala Glu Leu Val Arg Pro Gly  
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Thr Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Thr Asn  
 20 25 30

Tyr Trp Leu Gly Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp  
 35 40 45

Ile Gly Asp Ile Phe Pro Gly Ser Gly Asn Ile His Tyr Asn Glu Lys  
 50 55 60

Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala  
 65 70 75 80

Tyr Met Gln Leu Ser Ser Leu Thr Phe Glu Asp Ser Ala Val Tyr Phe  
 85 90 95

90

Cys Ala Arg Leu Arg Asn Trp Asp Glu Pro Met Asp Tyr Trp Gly Gln  
 100 105 110

Gly Thr Thr Val Thr Val Ser Ser  
 115 120

&lt;210&gt; 97

&lt;211&gt; 339

&lt;212&gt; DNA

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; EpCAM 5-10 VL

&lt;400&gt; 97

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 tgggtaccagc agaaaccagg gcagcctcct aaactgttga tctactgggc atccactagg 180  
 gaatctgggg tccctgatcg cttcacaggc agtggatctg gaacagattt cactctcacc 240  
 atcagcagtg tgcaggctga agacctggca gtttattact gtcagaatga ttatagttat 300  
 ccgctcacgt tcggtgctgg gaccaagctt gagatcaaa 339

&lt;210&gt; 98

&lt;211&gt; 113

&lt;212&gt; PRT

&lt;213&gt; artificial sequence

&lt;220&gt;

&lt;223&gt; EpCAM 5-10 VL

&lt;400&gt; 98

Glu Leu Val Met Thr Gln Ser Pro Ser Ser Leu Thr Val Thr Ala Gly  
 1 5 10 15

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser  
 20 25 30

Gly Asn Gln Lys Asn Tyr Leu Thr Trp Tyr Gln Gln Lys Pro Gly Gln  
 35 40 45

Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val  
 50 55 60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly<sup>91</sup> Thr Asp Phe Thr Leu Thr  
 65 70 75 80

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn  
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Asp Tyr Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Ile  
 100 105 110

Lys

<210> 99

<211> 15

<212> PRT

<213> artificial sequence

<220>

<223> standard linker

<400> 99

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<212> DNA

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<223> 4-7 VL BspEI FOR

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47

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<212> DNA

<213> artificial sequence

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<223> 4-7 VL GS15 REVs148

<400> 101

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# INTERNATIONAL SEARCH REPORT

In national Application No  
EPO, EP2004/005687

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K16/30 C07K16/28 A61K39/395 A61K16/46

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, EMBASE, BIOSIS, MEDLINE, Sequence Search

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

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Date of the actual completion of the international search

21 October 2004

Date of mailing of the international search report

04/11/2004

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Irion, A

## INTERNATIONAL SEARCH REPORT

Int'l Application No  
PCT/EP2004/005687

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A	----- RAUM TOBIAS ET AL: "Anti-self antibodies selected from a human IgD heavy chain repertoire: A novel approach to generate therapeutic human antibodies against tumor-associated differentiation antigens" CANCER IMMUNOLOGY IMMUNOTHERAPY, vol. 50, no. 3, May 2001 (2001-05), pages 141-150, XP002301913 ISSN: 0340-7004 the whole document	1-25

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PCT/EP2004/005687

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